PHYTOPLANKTON METHODS MANUAL

WITH SPECIAL EMPHASIS ON WATERWORKS OPERATION INTERNAL METHODS MANUAL

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Internal Methods Manual

Report Prepared By:

Limnology Section
Water Resources Branch
Ontario Ministry of the Environment

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Introduction

During the 1960's and 1970's the Biology Section of the former (OWRC) and Water Quality Branch M.O.E. provided a one week training course in algae identification and enumeration for water works personnel. During the 1980's our priorities were directed to other programmes and the training courses for water supply personnel were transferred to the Training, Development and Certification Section of the Human Resources Branch.

Algae Identification and Enumeration analytical services remained as a function of the current Aquatic Plant Unit, Limnology Section, Water Resources Branch. This service is provided to support water quality assessments for various Ministry funded programmes.

This manual replaces the Algae Identification and Enumeration Course Manual which was last printed in 1970. It has been modified to include taxonomic revisions and quantitative methodologies which have been adopted by the Limnology Section during the intervening years. It includes changes to sample preservation, sample preparation, and concentration techniques. Alternatives to quantification of algal biomass including biovolume measurements and some statistical measurements to assess counting accuracies have been added. Additional sections will be included in the future to document QA/QC procedures and computerized counting techniques which are now under development.

While the previous editions were in the form of lectures and laboratory notes and instruction, this edition will emphasize the documentation of the methodology. The sections describing laboratory demonstrations and hands-on practice have been included in this manual but are not an essential part of it.

ALGAE AND OTHER INTERFERENCE ORGANISMS IN WATER SUPPLIES

WHAT ARE ALGAE?

Algae are plants just as trees and grass are plants. They are green, they manufacture their food in the form of starches or oils by using the energy of sunlight and the nutrients they extract from the water. In the classification of plants, they are considered to be the most primitive groups, and some of the algal forms commonly found in water supplies are thought to be similar to the first life on earth. They are considered primitive because each cell is capable of carrying out the complete life history as no specialization has been developed into various tissues such as are found in the higher plants (stems, roots, leaves, seeds). All higher plants and animals are composed of millions of cells. Throughout the ages, certain cells of plants and animals have arranged themselves into specific organs (kidney, heart, liver, skin) which carry out a highly specialized function. In algae, each individual cell fulfils all such functions, i.e. excretion, respiration and reproduction.

VALUE OF ALGAE

The waterworks operator often looks upon algae as purely a nuisance as it clogs filters, imparts tastes and odours to the water supply, and causes growths on reservoir walls.

Algae are however, the basis of all life in water. On land, grass feeds the rabbit which in turn is eaten by the fox and while the fox does not eat grass, there would be no foxes if there were no grass. This is called the food chain and the basis of the food chain in water, is algae. Algae feed minute animals which in turn are eaten by minnows which in turn provide the food for larger fish such as bass and walleye. Thus if there were no algae there would be no fish. It can be demonstrated that fish production in a lake varies directly with the amount of algae that it produces and thus while algae may be a disadvantage in a water supply, they are not unnatural and are necessary for other uses we make of water. When the first humans start making long distance trips into space, the food they will consume will likely include algae grown in the space capsule.

SIZE AND DISTRIBUTION

There are several thousand different species of algae that live in the waters of Ontario. These range in sizes from a plant as much as four feet tall down to cells which are so small that they can barely be seen when magnified a thousand times in a microscope. In addition to living in the oceans, lakes and rivers, down to the depth where the light can penetrate, they also live on the damp soil on the face of glaciers, and in combination with fungi to produce the lichens we are all familiar with.

GROWTH REQUIREMENTS

Algae are very specific in their needs. The types that are characteristic of lakes are seldom found in streams and those which populate a lake in summer give way to other forms in winter. Some species can only live in very pure water and others are obligated to polluted situations and even sometimes to particular types of pollution. Factors governing the type and number of algae are environmental such as temperature, available light, and nutrient concentrations such as nitrates, phosphates, manganese, iron etc. No two species of algae are likely to have exactly the same requirements.

SIGNIFICANCE AND INTERPRETATION OF ALGAE

Algae are normal and constant inhabitants of nearly all natural surface waters. Wherever algae grow, there too will be found bacteria, fungi and various animals. These different organisms interact in the open waters to carry on a chain of life. The algae as green plants use the dissolved solids, nutrient minerals, water and carbon dioxide to grow and reproduce. Bacteria and animals feed on the dead or live organic algae. The bacteria in turn die, and their cells are broken down, thus eventually returning the elements to the mineral condition. If any part of this cycle were to be eliminated or altered, the resulting water might contain materials that would cause tastes and odours, or be otherwise undesirable as a water supply. The living creatures serve to stabilize the water and to degrade or decompose foreign material, such as pollution, that may enter the aquatic environment.

The more frequently observations on a raw water source are made, the greater the likelihood of noting the beginning of increased algal populations. Counts may vary at a few hundred cells per millilitre for an extended period, then climb perceptibly to a few thousand cells per millilitre within three to four days. Such increases may result from growth simulated by a change in the weather; by an increase of nutrients from sewage effluents, land drainage, precipitation, or applied fertilizers; or from planktonic masses drifting out from adjacent fertile tributaries.

Excessive numbers of one algal type may cause no end of trouble in the water treatment plants. It is therefore important for the plant operator to know what is in the water. No specific rules can be set down which will account for all local circumstances and it would be misleading to quote a number of plankton counts and imply that the consequences of each would always be the same.

Waterworks operators can learn a great deal about the general quality of the water by an examination of the phytoplankton populations. Certain generalizations can be made:

1. Severely polluted warm or hard waters tend to encourage blue-green algal forms or euglenoids.

2. Cold, clear waters generally favour diatoms and chrysophytes.

3. Green algae tend to be abundant during spring and late fall seasons.

Thus, one of the great benefits that can result from a systematic program of phytoplankton counting is to predict expected "blooms".

ALGAE PROBLEMS IN THE WATER SUPPLY SYSTEM

The operator can be faced with problems created by algae of several kinds. In all cases, they result from an over-abundance, but the numbers required to create this difficulty will vary.

1. Filter Clogging

The reduction of filter runs, caused by the coating on the surface of the filters with large numbers of these minute plants is probably the most common and serious problem that algae create for the waterworks operator. Certain waters at certain times of the year produce a great abundance of the filter clogging species and under the worst conditions may reduce the production of water through a filter to a point where there is hardly sufficient water to backwash. The lake diatoms such as Asterionella, Melosira, Synedra, Tabellaria, Fragilaria and Stephanodiscus are the most common trouble makers in this regard, but certain of the summer blue-green algal forms may also develop in sufficient numbers to reduce filter runs. One year, the diatom Melosira caused a great reduction in the normal filter runs at several waterworks along Lake Ontario.

2. Taste and Odour Production

Algae are capable of producing tastes and odours that will persist through treatment and cause consumer complaints. Bacteriological doses of chlorine often rupture the algal cell wall thus expelling the cell's waste material into the water supply system. Certain blue-green algae such as Anabaena, Aphanizomenon and Microcystis are well known for developing very foul "pigpen" odours in water. These blue-green forms collect in large masses sufficient to form water "blooms". The foul odour undoubtedly develops from products of decomposition as the algae begin to die off in large numbers.

Generally, different algae have been shown to cause different flavours and odours that have been variously described as pigpen, grassy, musty, cucumber, etc. Much of the difficulty of tastes and odours is the result of the decomposition products of rooted aquatic weeds. Raw water supplies that come from shallow, weedy lakes almost invariably have a continuous or intermittent problem with tastes and odours. This problem is usually most acute late in the fall when the ice-cover has just formed and again in the spring at the time of the break-up of ice-cover. Several softwater lakes in Ontario have recorded the odour-producing flagellate Synura

present in their waters. These algae in low numbers impart a perceptible cucumber-like odour to the water, and at later stages , a distinctly "fishy" odour.

3. **Growths in Reservoirs**

A third common problem that algae create in a waterworks operation is growths in reservoirs. Here the algae may grow attached to the walls where they form a heavy mass of material. This algae mass may be alive with crustaceans and insect larvae. Occasionally, one of these little animals may come through the taps and shake the confidence of the consumer in the purity of the supply. Algal growths in the reservoir may be of a free-floating type. These growths may impart tastes to the water. Probably the most common algae causing difficulty in reservoirs is one of the larger species called Chara. This algae grows to a height of two or three feet in a soft mud bottom and is typical of the cold, hard water commonly found in spring water sources. Where such water is collected and stored in an open reservoir this alga invariably grows and is difficult to control.

CONTROLLING ALGAE

There are two basic means of controlling algae and solving the problems that they create. One is by controlling the environment in such a way as to make it an unsuitable place for them to live, and the other is by treatment practices. The latter method is less satisfactory in the long run as it necessitates adding chemicals that are costly on either a continuous or intermittent basis. Controlling the environment is a more satisfactory means, though this is not always possible.

1. **Environmental Control**

The best method of this type of control is by selecting the best possible source of supply. A deep, cold lake rather than a shallow, warm productive lake should be chosen. In choosing a new supply, care should be taken to utilize water of low fertility as judged by chemical analyses and the algae population that it maintains. Routine biological and chemical samples should be obtained for at least one year previous to the installation of any waterworks facilities. Limnologists would then be able to determine the numbers and kinds of algae present and assess the suitability of the water. Where the municipality controls the land adjacent to the supply, care should be taken to keep out surface drainage and other possible nutrient sources. Run-off from buildings, domestic sewage and certain industrial wastes are rich in plant nutrients and should be avoided as only small amounts of these fertilizing substances can induce the development of high algae populations.

Another aspect of environmental control is to exclude the light by covering pre and post treatment reservoirs. The

easiest method is simply to cover the reservoir, although this is often not done and algae problems continue year after year. This may be achieved by a permanent cover such as concrete or a black plastic sheet spread over the reservoir. While the latter method has not been used, it would be effective in excluding light and be relatively inexpensive to apply. A second method of reducing light in pre-treatment reservoirs is to use activated carbon to induce an artificial turbidity. While this is only a temporary measure and must be repeated every four days, it has the added advantage of adsorbing taste and odours from water while in suspension and keeping the bottom accumulation sweet. Carbon can only be used in the raw water where the followingtreatment includes effective filtration.

2. Treatment Practices

a) Control of Filter-Clogging Algae

The obvious way to solve the problem of short filter runs is to remove the algae before they get to the filter. The common method of removing algae from raw water is the use of settling basins which may follow flocculation and pre-chlorination. Microstrainers are also being used to remove the algae before the water is filtered. A third method is to apply algicides to the raw water and thus remove them before the water enters the plant.

The principle of removing algae by flocculation and sedimentation involves trapping the algal cell in the alum floc and carrying it to the bottom with other unwanted solids from the water. When algae populations are very high they often hold the floc in suspension long enough for it to pass through the settling basins and onto the filters. This floc and the algae can be settled if weight can be added to the floc. A slurry of ordinary clay mixed and fed during the periods of difficult times will do much to get the operator over a short term period of difficulty. Increased dosages of alum and heavier pre-chlorination will also assist in alleviating short filter runs.Alternative methods of treatment, such as dissolved air floatation are used elsewhere in the world and are currently being investigated in Ontario.

b) Chemical Control

While there are many algicides sold today, only two are suitable for use in a domestic supply, namely copper sulphate and chlorine. The use of copper sulphate for aquatic nuisance control is no longer allowed in Ontario. However, other copper based compounds may be used provided a permit for application has been obtained from the Hazardous Contaminants Branch, Ministry of the Environment.

In applying chlorine, a rough calculation must be made of the volume of water being treated and the kilograms of chlorine required to satisfy the demand and still provide a residual of 1 ppm. The calculation is used as an initial guide, then followed by chlorine tests to provide the final adjustments. A similar calculation must be made for determining the amount of the copper product used but more care must be exercised as no simple test can be used as a guide. To do this, the surface area of the water (in metres) to be treated must be obtained together with the average depth of the water. When multiplied these two figures give the volume of water in cubic meters. The total kilograms pounds of water may then be calculated by multiplying the volume by 1000. As one part per million (ppm) equals 1 kilogram per million kilograms of water, the treatment of a reservoir with .5 ppm would require one half kilogram of chemical for each million kilograms of water.

Area x average depth x 1000 = kg of water in reservoir

1 ppm = 1 kg per million kg of water

The ideal time to apply chemicals is when the algae population is rising but before the condition becomes acute. If treatment is postponed until a very dense growth of algae occurs the sudden killing of this material and the subsequent decomposition may remove all the oxygen from the water causing it to go septic, kill the fish, and become foul-tasting. If the algal growth gets out-of-hand before treatment can be applied, half the reservoir should be treated first to reduce the population; after a week or so has been allowed for this material to decompose, the total reservoir area can then be treated.

c) Microstraining

Microstraining as a method of water treatment was introduced in Ontario several years ago. There are about six installations operating on municipal water supplies. The development of this means of filtration was made possible by the invention of an extremely fine wire mesh capable of removing such small particles as algae from the water and yet capable of passing high volumes of water. The principle of the microstrainer is simply a rotary screen where the raw water is fed to the inside and flows out through the screen material. The drum is about three quarters immersed and as it turns around, a jet of water is played on the surface of the screen and knocks down the accumulated solids and algae into a hopper and from there are carried to waste.

In water treatment the microstrainer has two uses:

(i) as pre-treatment for algae removal ahead of conventionals and filters,

(ii) as sole treatment for waters for the removal of algae and other extraneous material where turbidity is not a problem.

Microstrainers have proven very effective in extending the operating time of conventional sand filters during times of heavy algae "blooms". In one instance, runs of not less than 20 hours have been obtained where previously 6-hour runs in summer were not uncommon and as little as two hours were experienced. Where a microstrainer is used as a sole means of water treatment it should never be installed with the thought of reducing turbidity to safe levels. Where it has been used solely for the removal of algae and the protection against the variety of water fleas, insect larvae, leeches and aquatic worms, that commonly pass through unprotected water supplies, it has been found to be very satisfactory.

Some of the microstrainers installed in the province have been set up on an automatic control system and some are operated manually. The system used will depend on the individual plant. In general, they are easy to operate and require the normal lubrication and an occasional wash down. Over a period of time some permanent plugging of the screens will take place that is not backwashed by the water jet. When this occurs, the strainer must be drawn down and a 12% sodium hypochlorite solution applied directly to the fabric while the screen turns over slowly. It should be emphasized that concentrated chlorine solutions from a chlorinator or from chlorine powders are not effective in rehabilitating the screen capacity.

The reason for the sliming of the fabric is not well understood. The time between washing has varied anywhere between one day and six months. In one or two instances difficulties due to lessening of filter capacity over a period of one or two days have occurred. In all cases, the screens have been quickly rehabilitated with the hypochlorite wash and an investigation is now underway to obtain a continuous method of protection against this short-term loss of capacity.

d) Control of Algae-Caused Tastes and Odours

Several methods are commonly used in controlling tastes and odours; the feeding of activated carbon and, variations in the method of chlorination and more recently ozonation has been used in Europe and North America. Activated carbon is the most widely used treatment and is usually effective in controlling most tastes and odours, but on a continuous basis is somewhat expensive. Also, this method is somewhat messy. Another method of controlling tastes and odours is to alter the method of chlorination. Super-chlorination or break-point

chlorination may be helpful. These methods must be tested asthe algae may be killed leaving the decomposition products to make conditions worse. The use of chlorine dioxide or chloramine at various points of application in the plant may assist in controlling tastes and odours but this is an individual problem within each waterworks and can only be determined through experimentation.

IDENTIFICATION OF PLANKTON (GENERAL)

PLANTS

Plants are photosynthetic, i.e. in the presence of sunlight they can synthesize inorganic nutritional materials to provide for growth and reproduction. This is made possible by the presence of the pigment chlorophyll which imparts the green colour to all plants, large and small. While the proper classification of some of the single-celled forms of life is open to dispute among microbiologists because some have both plant-like and animal-like characteristics, a simple means of differentiation is to categorize all organisms which contain chlorophyll as plants. Sometimes other pigments are present in algae which impart a blue-green or brown colour to certain forms of these tiny plants because the chlorophyll is masked by these other pigments (e.g. blue-green algae - phycocyanin).

PHOTOSYNTHESIS

$$\text{Carbon Dioxide} + \text{water} \xrightarrow[\text{and Chlorophyll}]{\text{in the presence of Sunlight}} \text{starches \& sugars} + \text{oxygen}$$

This is an extremely simplified formula indicating what takes place as plants manufacture their food. A balanced condition is provided by the fact that animals breathe the oxygen produced by plants in order to metabolize their food and release energy for movement and other bodily activities, at the same time producing carbon dioxide which is essential to the plants.

FUNGI

Fungi are a somewhat specialized group which, for a long time, have been classed by most biologists as plants. However, they are distinctly different in that they do not possess chlorophyll and are unable to synthesize their own food. Fungi are able to secrete enzymes which change insoluble food to a soluble form which is assimilated and metabolized within the cells.

ANIMALS

Most animals ingest and break down solid food of organic origin although there are some unicellular forms which assimulate soluble food materials yhrough their cell membranes. They cannot produce their own food and never contain chlorophyll.

Planktonic forms of interest to us represent the Plant and Animal Kingdoms. Algae are the tiny plants, mostly microscopic in size, that are extremely influential in affecting water supplies in certain areas. In addition, there are both unicellular and multicellular animals which are encountered and which may also cause nuisances.

ALGAE

These tiny, chlorophyll-bearing plants may be single-celled or the cells may be grouped together in filaments or colonies.

Although there are quite a number of major groups of algae which are recognized by taxonomists, for purposes of simplification, the algae which are significant in water supplies may be grouped into four different types. These are as follows:

a) Blue-green algae
b) Non-motile green algae
c) Pigmented flagellates
d) Diatoms

THE CLASSIFICATION OF ORGANISMS

One of the first questions usually posed about an organism seen for the first time is: "What is it?" usually meaning, "What is its name?" The naming or classification of biological organisms is a science in itself (taxonomy).

The system of biological nomenclature is regulated by an international code. It is based on a system of groups and super groups, of which the foundation is the species. The categories employed are as follows:

The species is the foundation.

Similar species are grouped into genera (singular: genus).

Similar genera are grouped into families.

Similar families are grouped into orders.

Similar orders are grouped into classes.

Similar classes are grouped into phyla (singular: phylum).

Similar phyla are grouped into Kingdoms.

The scientific name of an organism is its generic name plus its species name. This is analogous to our system of surnames (family names) and given names (Christian names). The first letter of the generic (genus) name is always capitalized and that of the species name is written with a small letter. Both names should be underlined or printed in italics when used in a technical sense.

i.e Homo sapiens - modern man

Esox lucius - northern pike

A complete list of the various categories to which an organism belongs is known as its "classification". This may be written as follows for Phacus pyrum, a green flagellate:

Kingdom	-	Plantae
Phylum	-	Euglenophyta
Class	-	Euglenophyceae
Order	-	Euglenales
Family	-	Euglenaceae
Genus	-	Phacus
Species	-	pyrum

Algae which are found in water supplies are placed in four general groups as discussed in Sections 5 to 9. The most common taxonomic Classes of algae found in fresh waters are as follows:

1. Bacillariophyceae
2. Chlorophyceae
3. Chrysophyceae
4. Cryptophyceae
5. Dinophyceae
6. Euglenophyceae
7. Cyanophyceae
8. Prasinophyceae
9. Raphidophyceae
10. Xanthophyceae
11. Prymnesiophyceae
12. Phaeophyceae
13. Rhodophyceae

See Appendix 'A' for a more detailed discussion on the taxonomic classification of algae (Section 18). The first seven classes contain the most common taxa found in fresh water water supplies.

ANIMAL LIFE ENCOUNTERED IN WATER SUPPLIES

SINGLE CELLED ANIMALS

Protozoa

Unicellular forms of animal life are collectively referred to as Protozoa. Five major groups of Protozoa are recognized as follows:

1. Sarcodina - e.g. Amoeba, Arcella
2. Mastigophora - e.g. Bodo
3. Ciliata - e.g. Paramecium, Vorticella
4. Sporozoa - not of interest - occur as parasites in plants and animals.
5. Suctoria - e.g. Acineta

Representatives of the Sarcodina groups have pseudopodia, finger-like processes which develop and are constantly changing in shape and the animal literally "flows" as it moves along very slowly. Pseudopodia may also be extended to engulf food particles which are assimilated through the cell membrane of the animal into the protoplasm within the cell. Protoplasm is the jelly-like constituent of all plant and animal cells in which all of the basic life processes occur. The Mastigophora group is comprised of those forms which bear flagella (singular - flagellum). These are whip-like appendages which are used in movement. Flagellates move in corkscrew-like fashion. The possession of cilia is the outstanding characteristic of the Ciliata. Cilia are hair-like appendages which cover the body of the cell or are located at the anterior end of the animal. They are used in movement and in some cases for capturing food. Both free-swimming (Paramecium) and attached (Vorticella) forms of ciliates are often encountered. The Suctoria possess tentacles which are used to sting other organisms which pass nearby and to suck the contents from their victims.

MULTICELLULAR ANIMALS

Rotifers

These more complex animals have one or two crowns of cilia at the anterior end of the animal which resemble wheels. These cilia beat to permit movement and to create suction currents for drawing in food particles. The mastax is a powerful set of jaws which is clearly evident in the body of the animal which grinds up food with a hammer-like action.

Aquatic Worms

Small aquatic "sludgeworms" are related to terrestrial earth-worms. These tiny worms are able to withstand low dissolved oxygen conditions and are frequently observed as pink or red carpets on the bottoms of polluted streams. They are occasionally observed on the filter beds in water filtration plants.

Midge Larvae or Blood Worms

These segmented insect larvae are often vivid red in colour and are worm-like in character. They may be distinguished from the aquatic nematodes by the fact that they are somewhat thicker in diameter and microscopic examination may reveal hooks and breathing tubes.

Daphnia and Cyclops

These are tiny relatives of the common crayfish which feed on algae and are an important food item for small fishes. They appear as tiny white specks which move through the water with a jerky motion.

Scuds or Amphipods

These are somewhat larger crustaceans than the Daphnia and Cyclops, which may grow up to half an inch in length. They move smoothly through the water and their abdominal gills may be seen functioning when the animal is at rest.

Hydras

These tiny freshwater coelenterates have a columnar body usually 15 to 20 mm. in length with a ring of tentacles around one end of the body, which are used to paralyse and capture prey. Hydras have been known to live on the walls of filter beds and to sometimes clog filters when they reach particularly high numbers in lake waters.

Moss Animals or Bryozoans

Various treatment plants along Lake Ontario and Lake Erie have reported the presence of free-floating statoblasts of Pectinatella. The statoblasts are reproductive bodies from which larger jelly-like colonies develop. Other bryozoans form moss-like brownish mats which are attached to the bottoms of lakes or streams.

TERMINOLOGY USED IN THE DESCRIPTION OF PROTOZOA

Pellicle - the cell membrane which determines the shape of the animal and encloses the inner components of the cell.

Protoplasm - the jelly-like substance which is contained by the pellicle in which the animal's life processes function. The protoplasm is divided into a clear outer portion called the ectoplasm, and a granular inner mass, the endoplasm.

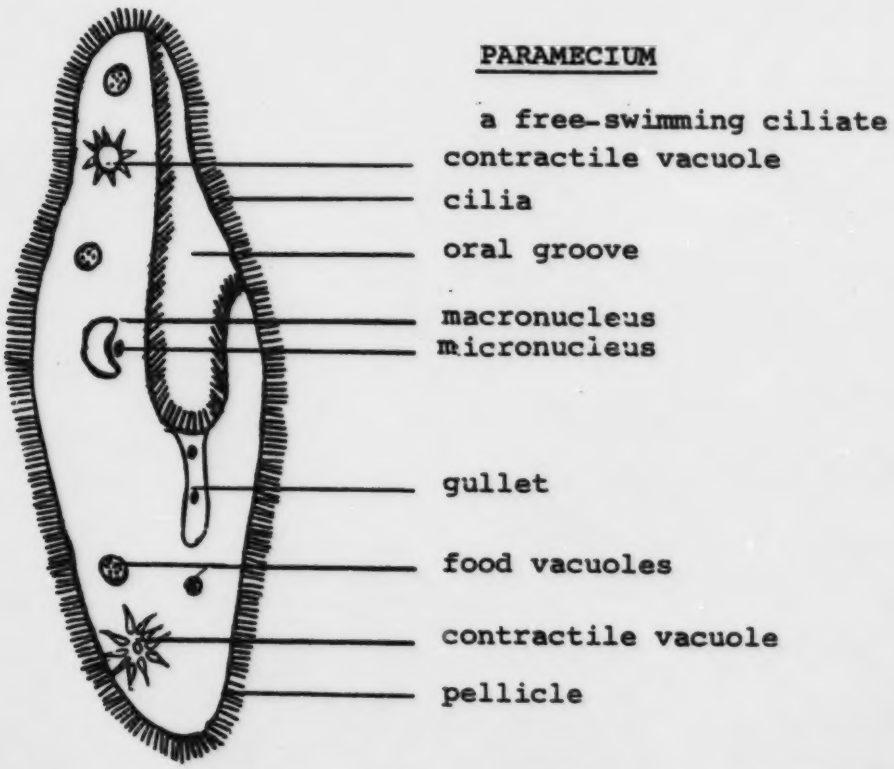
Macronucleus - the larger nucleus which governs bodily activities within the cell. Not present in Sarcodina.

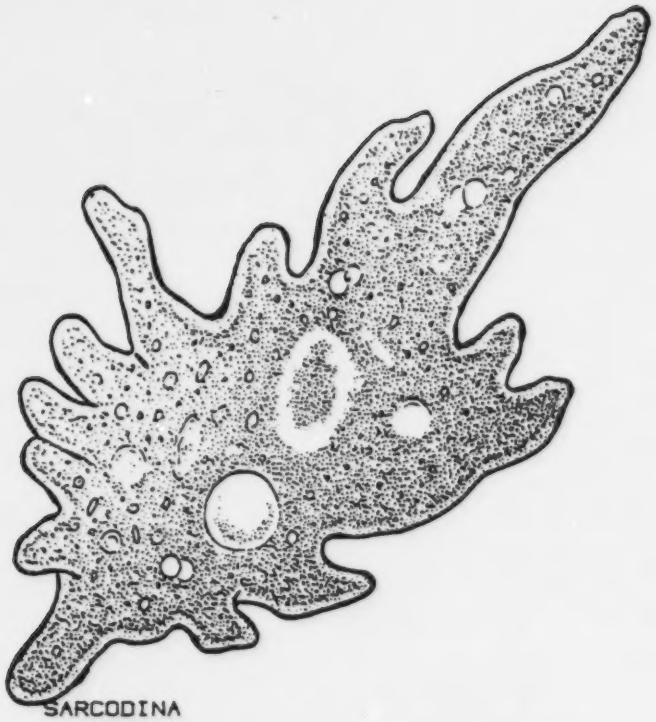
Micronucleus - the smaller nucleus which is involved in reproduction.

Contractile Vacuole - a relatively large, clear structure which is responsible for gathering and excreting water from the cell.

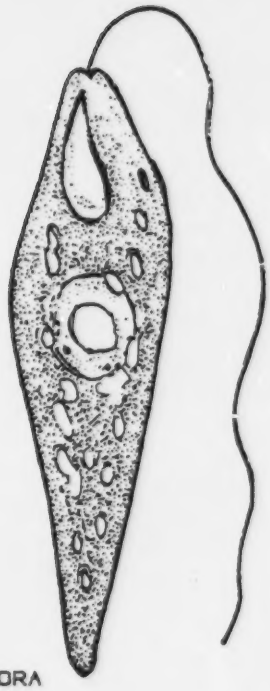
Food Vacuoles - structures in which food is being broken down by enzymic action.

Oral Groove - present in ciliates - opening lined with cilia into which food particles are drawn.

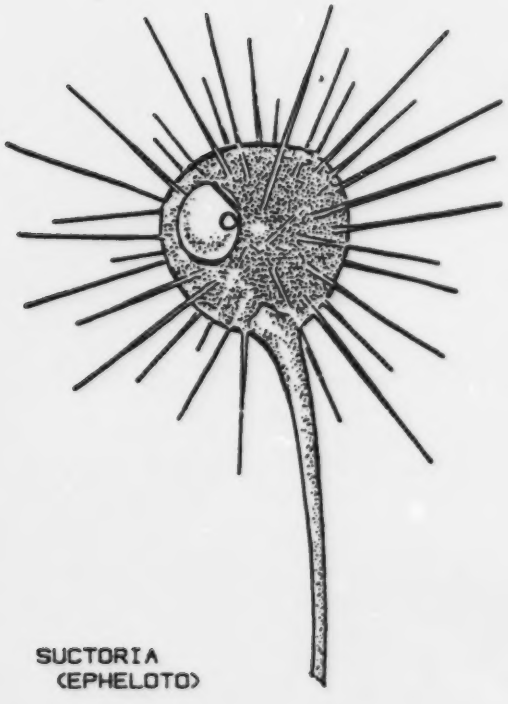




SARCODINA
(AMOEBA)



MASTIGOPHORA
(EUGLENA)



SUCTORIA
(EPHELOTO)

CILIATA
(VORTICELLA)

LABORATORY - FAMILIARIZATION WITH MICROSCOPE AND LEARNING TO RECOGNIZE ANIMAL PLANKTON

Objectives:

1. For beginners to learn how to use a microscope.
2. To learn counting and to associate these with basic animal types.

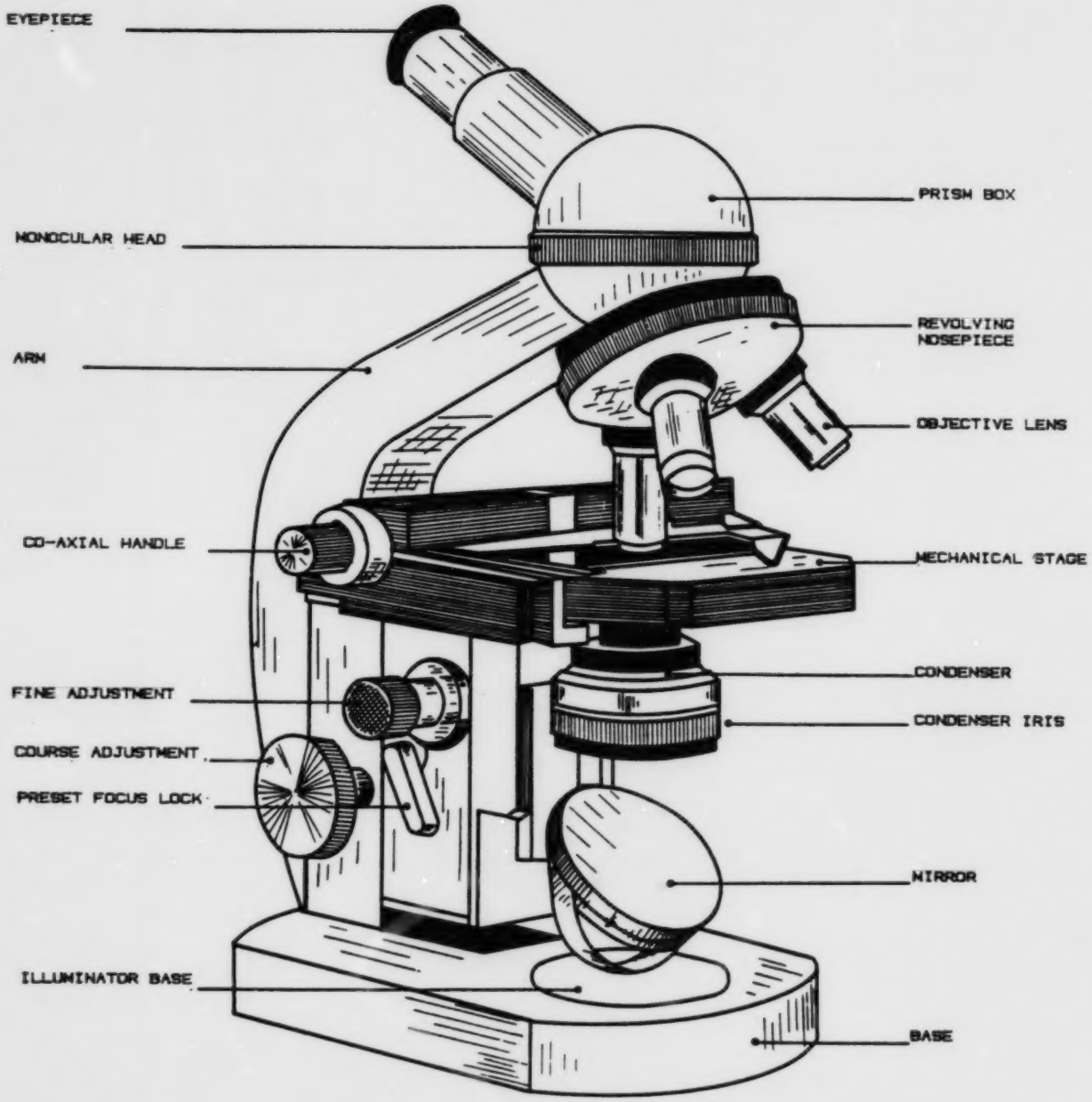
USE OF THE MICROSCOPE

1. Note the major parts - base, light source, condenser(s), stage, objectives, eyepiece, course and fine focus controls, and co-axial control to centre slide on stage. (See diagram p.4-2)
2. The bottom left-hand corner of the Sedgewick-Rafter cell is centred under the objective. Note how the image appears to the eye - upside down, and on the opposite side.
3. The magnification achieved is the product of the power of the eyepiece and of the objective. A 10X eyepiece and a 20X objective provides a magnification of 200X.
4. Practice focusing with the objective fixed over the edge of the Sedgewick-Rafter cell to gain an idea of the depth of field at 100X. (10X eyepiece and 10 X objective).
5. Practice moving the slide on the stage using the co-axial handle - forward and back, from left to right and vice versa.
6. Prepare a "wet mount" using a Daphnia from the collection of living materials and study it at 100X. Make a sketch of the organism on the blank sheet provided at the end of this section for this purpose.

IDENTIFYING ANIMAL PLANKTON (see next page)

Study and sketch as many of the living specimens as time will permit. Identify these using reference books available and with the help of the instructors. Methocel (methyl cellulose) which will slow the organism down (is available for you) so that they may be studied more easily. Make a tiny ring on the slide using the Methocel and place a drop of the sample containing the specimens inside the ring.

THE COMPOUND MICROSCOPE



Protozoa

- Single-celled organisms which may be seen as individuals or in colonial form. Have flagella, cilia or pseudopodia. See page 3-4 for types. Most forms lack the colour pigments which are present in algae.

Amoeba

ref. #2

Micro-invertebrates

Porifera

- Fresh-water Sponges - are usually seen as a spongy growth on any stable substrate such as submerged twigs and intake screens. Microscopically they consist of great numbers of needle-like spicules which are interwoven to hold together a non-cellular matrix.

ref. #1

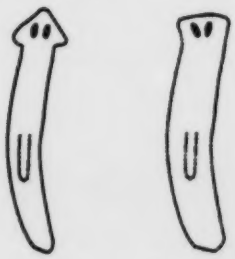
Coelenterata

- Hydra - an elongated cylinder with a basal attachment and a ring of tentacles at the distal (free) end.
- The tentacles surround the mouth and direct food to the interior hollow tube of the body.
- Daughter colonies called Buds may be present on the sides of the parent cell.
- Limited locomotion is achieved by summersaulting or gliding along the substrate.

ref. #2

Turbellaria

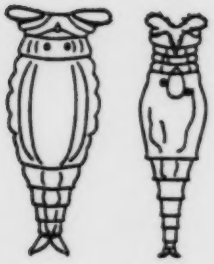
- Flatworms - elongated flat microscopic worm. Slightly larger than protozoa.
- No body segmentation. Eye spots may be seen at one end which distinguishes the head.
- Head may be pointed, triangular or flattened.
- Oral cavity and anus are one and the same, in the mid-region of the body.



Dugesia Planaria

ref. #2

Rotatoria



ref. #2

- Rotifers - Multicellular microscopic animals.
- Highly organized internal structure including mouth parts, mastax, digestive tract and circulatory system.
- Two wheel-like crowns of cilia may be seen at the anterior end of the body.
- Some species have a forked toe, others have a single posterior foot.
- Some have a hard shell-like cuticle or lorica.
- Others have a flexible, telescopic trunk-like body.
- The crown of cilia sort out the food to be eaten.
- The mastax consists of two muscular jaws which operate like hammers which macerate the food.

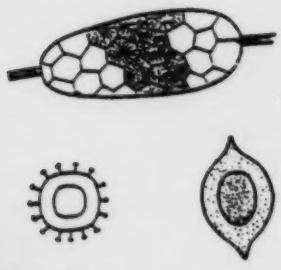
Nematoda

Nematodes

ref. #2

- Roundworms - round slender worm-like form - but lack segmentation.
- Highly organized internal structure i.e. digestive tract, reproductive organs (not evident in flat worms).
- Body diameter and length remains constant.
- Moves by continuous thrashing about of the body.
- Most individuals visible to the naked eye.

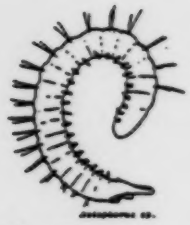
Bryozoa



ref. #1

- Moss Animalcules - in the water they appear as a large gelatinous mass which may have a brown or green colour.
- Individuals coat the surface of the gelatinous mass and microscopically appear like cylindrical branched tubes with a crown of tentacles at the tips of the branches.
- Statoblasts or resting cells are frequently seen on old colonies and are round or oval in shape with a border of anchor-like projections. Statoblast visible to naked eye.

Annelida



ref. #1

- Oligochaeta - commonly called aquatic earthworms or sludgeworms.
- These are true segmented worms with setae on each segment. Digestive tract and mouth parts are evident on microscopic examination.
- Visible to the naked eye.
- Usually associated with polluted conditions.
- Hirudinea - Leeches - are dorsalventrally flattened and visible without aid of microscope.
- Sucker type mouth.
- Muscular body is capable of contracting or expanding greatly.
- Eggs and cocoons may show up in water samples.

Micro-crustacea

Cladocera

ref. #2

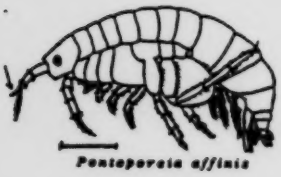
- Daphnia or Water Fleas - are usually visible to the naked eye as little white specks.
- Single eye is apparent on microscopic examination.
- One pair of branched antennae are used in locomotion (appears as a hopping or jumping motion).
- Organism is enclosed in an outer skeleton or carapace.
- Highly organized internal structure and appendages are present.

Copepoda

ref. #2

- Cyclops - adults visible to naked eye.
- Large branched antennae and highly branched swimming legs used for locomotion.
- Single eye apparent.
- Exoskeleton in form of a segmented and tapered shell.
- Egg sacs may sometimes be seen attached to abdomen.

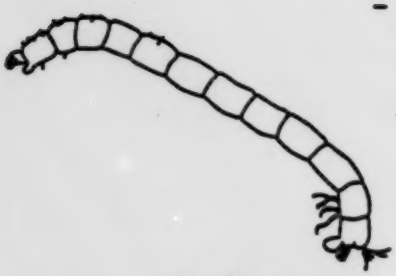
Amphipoda



ref. #4

- Scuds or side-swimmers are large enough to be seen with the naked eye.
- Body is laterally compressed and the two eyes are visible.
- There is a pair of appendages for each body segment, some of which are adapted for feeding, breathing, locomotion or reproduction.
- These organisms closely resemble their larger relative, the crayfish.

Insecta



Tendipes x 7

ref. #2

- Tendipedidae - Midge larvae are commonly called Blood Worms due to their bright red colour. They are not worms but the aquatic larval stages of true insects.
- They have segmented bodies with differentiated head and caudal segments.

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1) Eddy, S., and A.C. Hodson. 1961. Taxonomic Keys to the Common Animals of the North Central States. Burgess Publishing Company, Minnesota, U.S.A.

2) Moncrief, B. 1990. Algae Identification and Enumeration. Training, Development and Certification Section, Ontario Ministry of the Environment, Toronto, Ont.

3) Pennak, R.W. 1978. Fresh-water Invertebrates of the United States, 2nd Edition. John Wiley & Sons, New York, U.S.A.

4) Ward, H.B. and G.C. Whipple. 1959. Fresh Water Biology, 2nd Edition. W.T. Edmondson (Ed.) John Wiley and Sons, New York, U.S.A.

CHARACTERISTICS OF MAJOR TYPES OF ALGAE

Algae, which are important in water supplies, may be placed in four general groups, the blue-green algae (Cyanophyceae), the green algae (Chlorophyceae), the diatoms (Bacillariophyceae) and the pigmented flagellates (Dinophyceae, Cryptophyceae, Chrysophyceae and some Chlorophyceae and Euglenophyceae). These four groups follow the format of Palmer's "Algae in Water Supplies" (1962).

1. Blue Green Algae (Cyanophyceae)

These are the simplest forms of algae and are best described by what they do not have.

a) They are the only algae in which the pigments are not localized in definite bodies but dissolved throughout the cell. Blue, red, or other pigments are present in addition to chlorophyll thus giving the cells a bluish green, yellow or red colour, at least en masse.

b) No nuclear membrane is apparent.

c) No flagella (whip-like appendage to allow movement) is present.

d) Blue-greens tend to achieve nuisance concentrations more frequently in the warm summer months and in the richer waters.

e) Vegetative reproduction, in addition to cell division, includes the formation of "hormogones", or short specifically delimited sections of trichomes (filaments).

f) Some species have specialized spores which carry the algae through periods when unfavourable environmental conditions are encountered. Spores of three types are encountered:

   i) "Akinetes" are usually long, thick-walled resting spores.

   ii) "Heterocysts" appear like empty cell walls, but are actually filled with protoplasm and have occasionally been observed to germinate.

   iii) "Endospores", also called "gonidia" or "conidia", are formed by repeated division of the protoplast within a green cell wall.

g) Many species of blue-greens have a definite gelatinuous sheath or envelope surrounding each cell, colony or filament.

h) There are unicellular, colonial and filamentous species of blue-greens.

i) Examples: Anabaena (filamentous)

Microcystis (colonial)

Oscillatoria (filamentous)

Chrococcus (single celled or colonial)

2. Non-Motile Green Algae (Chlorophyceae)

The non-motile green algae constitute another heterogenous assembly of unrelated forms. In this classification system, the yellow-greens and desmids are included with the non-motile greens.

a) They have a well-defined nucleus.

b) They have well organized chloroplasts. These chloroplasts contain chlorophyll. The shape and position of the chloroplast is often distinctive. Pyrenoids are a distinctive characteristic of some members of this group.

c) They lack flagella or other locomotor appendages.

d) They have a semi-rigid cell wall.

e) There is an extreme structural variation among members of this group.

f) Some types tend to occur as a general planktonic mass or "bloom", often in combination with two or more species. Some examples are: Sphaerocystis (colonial), Pediastrum (colonial), Scenedesmus (colonial), and the desmid Closterium (single-celled).

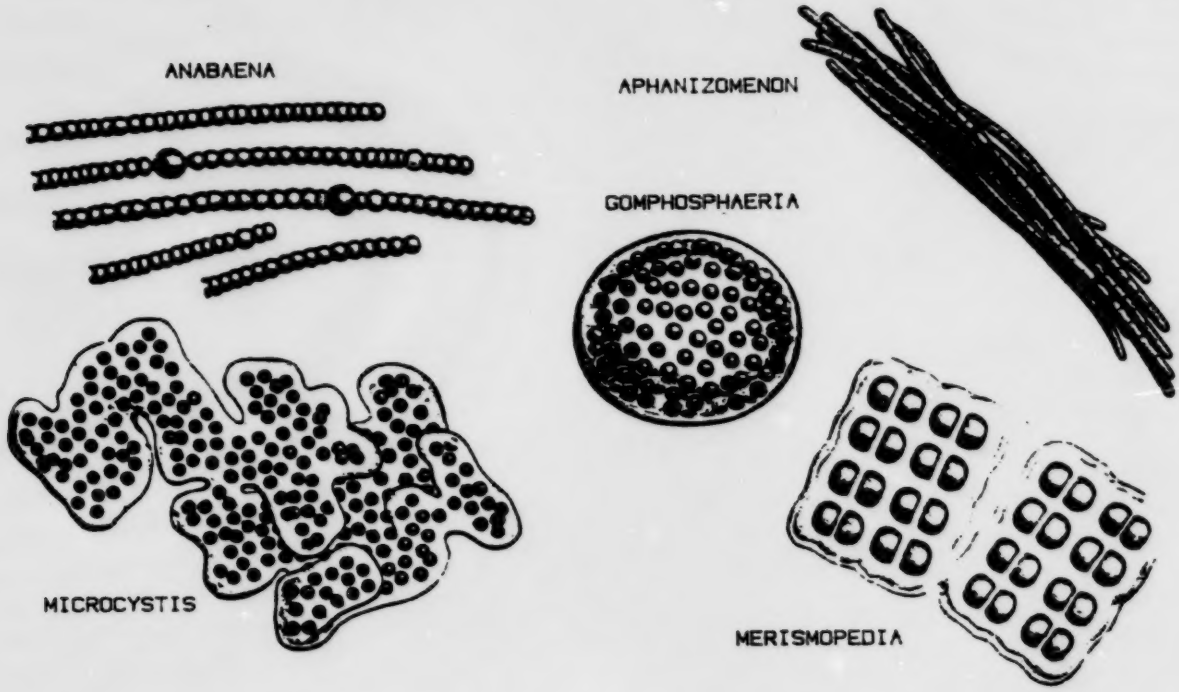
g) Thread-like (filamentous) algae may form masses or blankets, cutting off light, and reducing water circulation. They also add considerably to the total mass of organic matter. Some examples of this type are: Spirogyra, Cladophora, Oedogonium, and Chara.

3. Pigmented Flagellates

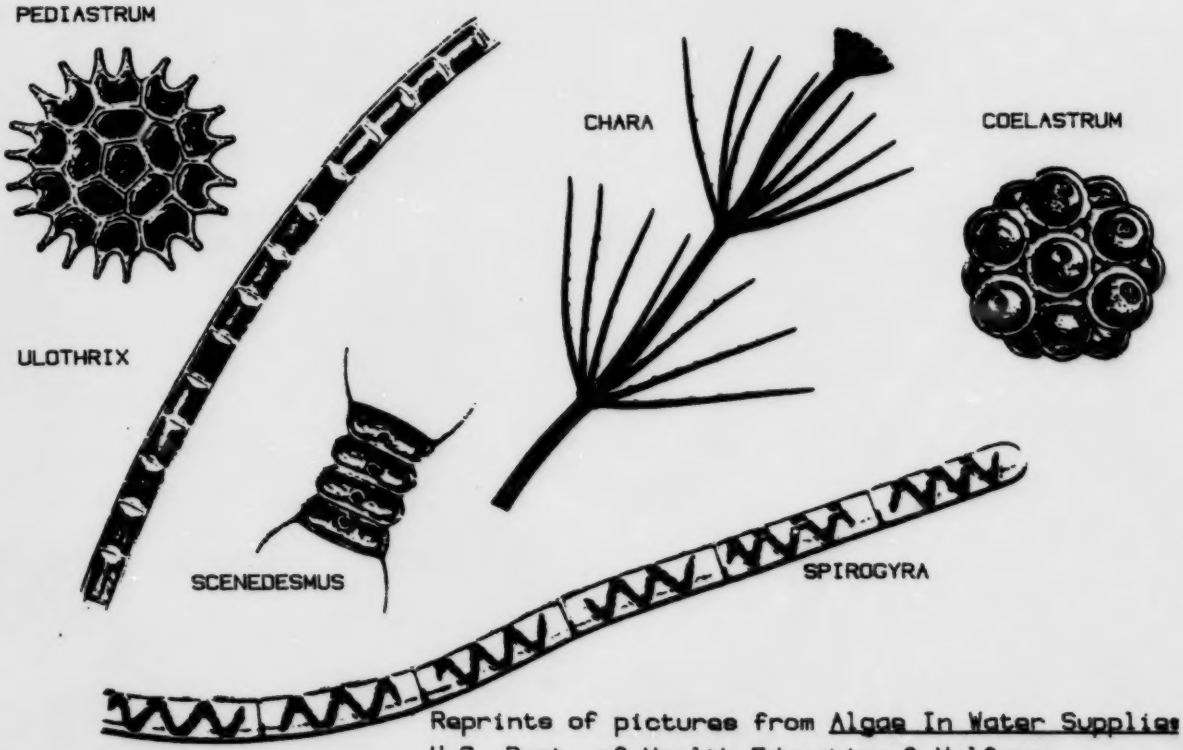
The "pigmented flagellates" (in contrast to the non-pigmented or animal-like flagellates) are a heterogeneous collection of motile forms from several different algal groups (Dinophyceae, Cryptophyceae, Chrysophyceae, Euglenophyceae, and motile Chlorophyceae).

a) They have one or more flagella per cell for movement.

BLUE GREEN ALGAE



NON-MOTILE GREEN ALGAE (Including Filamentous Types)



Reprints of pictures from Algae In Water Supplies
U.S. Dept. of Health Education & Welfare

b) A thin membrane is usually present surrounding each cell.

c) There is a well-defined nucleus.

d) A light sensitive eye-spot is usually present.

e) The chlorophyll is contained in one or more distinctive chloroplasts. In addition to chlorophyll, other pigments may be present in the chloroplasts.

f) Non-motile life history stages may be encountered in some forms.

g) Masses of stored starch called pyrenoid bodies are often conspicuous in certain genera of this group.

h) Examples: Euglena (single-celled)

Synura (colonial)

Chlamydomonas (single-celled)*

Dinobryon (single-celled or colonial)

Volvox (colonial)*

4. **Diatoms (Bacillariophyceae)**

a) In appearance they are geometrically regular in shape. The presence of a brownish pigment in addition to chlorophyll gives them a gold to greenish colour.

b) Motile forms move slowly in a distinctive hesitating progression.

c) The most distinctive structural feature is the two-part shell (frustule) composed of silicon dioxide (glass).

i) One part fits inside the other as two halves of a pill box, or a petri dish.

ii) The surface of these shells are sculptured with minute pits and lines arranged with geometric perfection.

iii) The view from the side is called the "girdle view", that from above or below, the "valve view".

d) A nucleus is present in all diatom cells.

e) There are two general shapes of diatoms, circular (centric) and elongate (pennate). The elongate forms may be motile, the circular ones are not.

f) Diatoms may be single-celled, colonial or filamentous.

g) Examples: Navicula (single celled)

Melosira (filamentous)

Fragilaria (colonial)

Cyclotella (single celled)

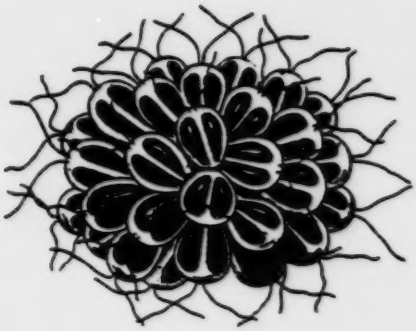
NOTE 1: For a summary of the four main algal groups, see Page 8 of Palmer's "Algae in Water Supplies" or Palmer's "Algae and Water Pollution". (See references section)

Note 2: Reprint of Algae on Page 5-3 and 5-6 are with the permission of U.S. E.P.A.

PIGMENTED FLAGELLATES

DINOBRYON

RHODOMONAS



SYNURA

CHLAMYDOMONAS

PERIDINIUM

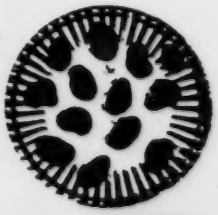
EUGLENA

DIATOMS

SYNEDRA

ASTERIONELLA

CYCLOTELLA



MELOSIRA

TABELLARIA

NAVICULA

FRAGILARIA

Reprints of pictures from Algae In Water Supplies
U. S. Dept. of Health Education & Welfare

BLUE-GREEN ALGAE - CYANOPHYCEAE

1. What are the Blue-Green Algae?

The blue-green algae (Cyanophyta) comprise that large group of microscopic organisms living in aquatic or moist habitats, carrying on photosynthesis and having differentiation of cells. Thus blue-greens are a little more complex than bacteria and simpler than all other plants called algae.

2. Characteristics

a) The presence of the blue-green pigment, phycocyanin, produces the blue-green colour which is characteristic of members of this group. Chlorophyll is present in addition to phycocyanin and is distributed throughout the cell rather than being contained in chloroplasts.

b) Some blue-green forms are gelatinous masses of various shapes floating in water. Others, microscopic in size, grow in great numbers so as to colour the water in which they live. Structurally their cells are similar to bacteria. Their protoplasts may be sheathed or embedded in gelatin, making them slimy.

c) There are no organized nuclei in the cells of the blue-green algae, no vacuoles, flagella or other locomotion appendages.

d) These algae reproduce by asexual means such as fission (simple cell division) or by fragmentation (filamentous forms). In certain forms such as Oscillatoria, specialized separation discs may form in a portion of the filament and the section between these two discs, called a hormogone, will break away to form a new filament.

e) Specialized cells are present in some species, such as akinetes, endospores and heterocysts. Akinetes and endospores may carry the algae through periods when unfavourable conditions are encountered. New filaments may sometimes be formed from heterocysts in some filamentous species such as Anabaena and Aphanizomenon.

3. Significance of Blue-Greens

They have both positive and negative economic significance. Because they can convert radiant energy into chemical energy, they are producers forming a first link at the base of the food chain. Because many very intricate nutritional relationships exist among the myriads of organisms, it is difficult to know the value of the blue-greens. When a "bloom" of blue-green algae develops, the algae sometimes drift into bays or along beaches where it decomposes. As

decomposition takes place, the mass of algae becomes unsightly, and creates foul odours and even toxins for some species. The decomposing algae are buoyed up to the surface of the water because "pseudovacuoles" or pockets of gas develop. There is usually a change to a yellowish colour as the algae degrade.

Some blue-green forms are able to clog pipes, filters and intake screens.

Some blue-greens live in association with other organisms as symbionts. Still others are found in polluted waters, because they are able to exist in habitats poor in oxygen. The growth of these kinds of algae under such conditions tends to make a polluted condition worse.

4. **Examples of Blue-Green Algae**

Most species of blue-greens may be placed into two major groups, the non-filamentous (coccoid) forms and the filamentous forms.

Microcystis

a) Colonies of Microcystis are always free floating.

b) There is a great variety in size and shape of the colonies of this genus (spherical or irregular, microscopic or macroscopic).

c) The gelatinous matrix surrounding colonies of Microcystis may be extremely transparent and is easily broken up on preservation.

d) Individual cells of this genus frequently contain pseudovacuoles.

e) Anacystis may include some Microcystis, Aphanothece and Gloeothece in some texts.

Anabaena

a) Filaments of Anabaena may occur singly, in irregular colonies, free floating or in a delicate mucous matrix.

b) The trichomes have practically the same diameter throughout and often form straight, spiral or irregularly twisted loops.

c) The vegetative cells of Anabaena are spherical or bowl-shaped, rarely cylindrical and never discoid.

d) Heterocysts are present which are the same shape but are slightly larger than the vegetative cells. These cells are usually clear in colour in this genus.

e) The akinetes of Anabaena are larger than the vegetative cells and tend to be sausage-shaped and densely pigmented.

f) This genus may produce an undesirable grassy, mouldy or septic odour.

g) Anabaena may be distinguished from Nostoc by the lack of a firm gelatinous envelope.

Aphanizomenon

a) Generally, cells of Aphanizomenon are smaller than in Anabaena, and trichomes are straight with cylindrical akinetes in evidence. The trichomes may be laterally joined into loose macroscopic free floating bundles which look like sheafs of wheat.

b) Cells are cylindrical or bowl-shaped and longer than they are broad.

c) Heterocysts of this genus generally do not occur at the end of the filament.

d) The akinetes are cylindrical and relatively long.

e) Aphanizomenon often imparts a grassy or nasturtium-like odour to the water.

Oscillatoria

a) Filaments may occur singly or are interwoven to form mats of indefinite extent.

b) Filaments are unbranched, cylindrical, and without sheaths.

c) Oscillatoria moves with a slow oscillating movement when living.

d) Species with narrow filaments have long cylindrical cells while those with broader filaments have short broad cells.

e) Oscillatoria has no akinetes or heterocysts. Hormogonia (short filaments) are formed and break away (fragmentation) as a means of reproduction.

f) Species of Oscillatoria may be readily distinguished from Lyngbya by the absence of a sheath.

g) Oscillatoria produces a distinct earthy odour which becomes septic in dense quantities.

Nodularia

a) Vegetative cells, heterocysts, and even the akinetes are broader than long.

b) Trichomes are practically the same diameter throughout.

c) Sheaths are usually distinct, fairly firm, and with a single trichome.

Lyngbya

a) This is similar to Oscillatoria but has a firm relatively thin, hyaline to yellowish-brown, homogeneous or lamellated sheath which encloses but a single trichome and generally projects for some distance beyond it.

b) The filaments are cylindrical and either straight or twisted into regular spirals.

c) The filaments may be grey, pale to bright blue-green, or variously coloured.

Merismopedia (Agmenellum in some texts)

a) The cells are arranged in flat plates in a gelatinous envelope. The cells are regularly arranged in vertical and transverse rows.

b) Small colonies are usually perfectly flat; large colonies, although one cell in thickness, are usually more or less bent and distorted.

Gomphosphaeria

a) Colonial form, oval or globular in appearance.

b) Cells usually arranged in a single layer about the periphery of the clear mucilaginous envelope and joined to strands which radiate out from the centre of the sphere. Some cells may appear closer to centre of colony as strands are not all equal in length.

c) Individual cells may be spherical or heart-shaped. Heart shaped formation is caused by fission process by which cells divide.

d) Cells contain a homogenous chloroplast which gives it a bright blue-green colour which may change to brown or grey with the increase in the presence of pseudovacuoles. Should be compared to Coelosphaerium.

LABORATORY - THE IDENTIFICATION OF BLUE-GREEN ALGAE

1. Using a taxonomic key* endeavour to determine the genus to which the sample of algae, provided by the instructor, belong. Even if you know the algae, follow through the key for practice.

2. Examine the preserved materials to become familiar with the genera available and terminology related to classification. Make sketches for future reference.

3. Examine the prepared slides Aphanizomenon, Anabaena and Microcystis under both low and medium power. Note the heterocysts and akinetes in Aphanizomenon and Anabaena.

* a) "Algae in Water Supplies" by C. Mervin Palmer

b) "Algae and Water Pollution" by C. Mervin Palmer

c) "Standard Methods" 16th ed. 1985 APHA

d) "How to know the Fresh Water Algae" by G. W. Prescott

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NON-MOTILE GREEN ALGAE - CHLOROPHYCEAE

FILAMENTOUS GREEN ALGAE

Filamentous green algae may be several millimetres or even up to a meter in length. In many cases they are not found as isolated filaments but develop in large aggregations to form floating or attached mats or tuffs. The attached forms are generally capable of remaining alive after being broken away from the substrate. Included in this group are some of the most common and most conspicuous algae in fresh-water habitats. A few of them have been given common names such as pond silk, green felt, frog-spawn algae, and stoneworts.

1. Characteristics

a) These algae are composed of cells held together end to end in filaments which may either be attached or free floating. In some green algae, the filaments are branched and in others they are not. Gelatinous envelopes are present in some species. Filaments may or may not taper towards the tips.

b) Each cell is either a short or long cylinder with a distinct wall, and contains a nucleus which is seldom readily apparent.

c) A chloroplast (plastid) is the most outstanding structure present and contains the pigment chlorophyll. The chloroplasts which are essential for food production vary in size, number, and shape in different algal forms. They may be "parietal" i.e. they lie close to the outer cell wall; others are "axial" i.e. they extend through the central axis of the cell. Some chloroplasts, as in Spirogyra and Zygnema are very distinctive and render identification possible on this feature alone.

d) Clear areas of cell sap ("vacuoles") are generally present in the green algal cells.

e) Attached green filaments have the basal cell developed into a "hold-fast cell" (hapteron).

f) Reproduction in filamentous green forms may take place by several methods:

i) Fragmentation of filaments may occur.

ii) Many kinds reproduce sexually, often with specialized gamete-forming cells.

iii) Zoospores (motile) and aplanospores (non-motile) are common.

iv) Cell division may occur in all cells or in certain selected ones.

2. Significance of the Filamentous Green Algae

a) Filamentous greens may promote development of animal plankton in storage reservoirs and lakes by providing the proper habitat conditions for micro-crustaceans such as Daphnia, Cyclops, etc.

b) Filamentous green algal forms may clog filters and intake screens at water treatment plants.

c) Foul odours may develop where these algae are washed ashore (e.g. Cladophora problem - Lake Erie and Lake Ontario). Large masses of Cladophora may wash ashore where it decomposes; thus interfering with the recreational values of the land.

d) Filamentous greens have been known to foul fishing nets (i.e. Cladophora - Bay of Quinte).

e) Green algae may produce a slime which interferes with some industrial uses of water such as in paper manufacturing and in cooling towers.

f) Green algal forms, along with other algae help to purify streams and maintain a favourable oxygen balance.

g) Some green algae are useful as indicators of water quality in relation to pollution.

3. Examples of Filamentous Green Algae - Unbranched Forms

Spirogyra

This plant is characterized by the presence of spiral chloroplasts which make up a large proportion of the contents of the cell. Pyrenoids, which are centres of starch formation, are arranged along the chloroplasts.

Ulothrix

a) This algae has a single parietal chloroplast in each cell. The curled edges of the chloroplast are usually evident. Sometimes confused with diatom Melosira.

b) Some species have cylindrical cells and others have cells shorter than they are wide.

Mougeotia

a) The presence of a plate-like axial chloroplast signifies members of this genus. The chloroplast sometimes shifts its position so that it appears as a narrow ribbon.

b) Chloroplasts of narrow cells have two, three, or more pyrenoids arranged in a linear series; chloroplasts of broad cells have several irregularly arranged pyrenoids.

c) Depending upon the intensity of illumination, the chloroplasts lie at right angles to, or parallel with, the incident rays of sunlight.

d) Chloroplasts of several successive cells usually have the same orientation, but sometimes the chloroplast of a single cell is so twisted that opposite ends are at right angles to each other.

e) The cell contains a single nucleus midway between the poles, and it lies flattened against the chloroplast.

Zygnema

A pair of star-shaped chloroplasts signifies members of this genus. A large central pyrenoid is always present in each of the chloroplasts. Application of Lugol's iodine solution facilitates observation of the chloroplast.

Oedogonium

a) Sterile specimens of Oedogonium may be recognized by the unbranched filaments of cylindrical cells. Certain cells have transversely striate walls at the distal end.

b) The basal cells of a filament is modified to form a holdfast, and the apical cell is usually broadly rounded.

c) Cells of Oedogonium are sometimes slightly enlarged at their ends, and have straight sides.

d) Cell division is either terminal or else intercalary and it may take place in any cell but the basal one. Where cell division has occurred by fragmentation, ring scars are left on the older cell at the point of detachment.

e) The cells are uninucleate and have a single reticulate chloroplast completely encircling the protoplast.

f) The chloroplast usually have many pyrenoids, one at each of the larger intersections in the reticulum.

4. **Branched Forms**

Cladophora

a) This genus is characterized by a spreading branching effect with its cells generally cylindrical in shape. It is a relatively large microscopic form. Branching may be alternate, opposite or dichotomous.

b) The ends of filaments taper very abruptly, with only the terminal cell being involved.

Stigeoclonium

This genus resembles Cladophora somewhat, but the tapering off of filaments is more gradual, involving two or more cells.

Chaetophora

This genus resembles Stigeoclonium but is surrounded by a gelatinous matrix.

5. **Specialized Forms**

Chara

These are large macroscopic plants which grow erect with stem-like branches which are arranged in whorls and bear forked leaves. Chara usually feels rough because lime is encrusted on it.

Hydrodictyon

This alga form a polygonal network of cells and is commonly called "water-net" on this account.

NON-MOTILE GREEN ALGAE - COCCOID

The coccoid algae are those that exist as free-floating planktonic units. Some non-motile coccoid forms tend to grow in masses or mats of material, either attached or free-floating.

1. **Characteristics**

a) Since green forms contain a good deal of starch, a deep purple colour will be produced when treated with Lugol's iodine solution.

b) Cells of some green species often link together to form coenobes. A coenobe is a colony of cells which does not multiply during the life of the colony although the cells do increase in size.

c) There is a great variety in size and shape among the members of this group. Cells or colonies may be round, irregular and often are ornate.

2. Significance of Coccoid Green Algae

a) Coccoid green algae may cause raw water supplies to be odorous and may contribute to filter clogging.

b) These algal forms may assist in maintaining a favourable dissolved oxygen balance in the water.

c) These algal forms are important items in aquatic food chains.

3. Examples of Coccoid Green Algae

Chlorella

a) Cells are small and generally spherical in shape.

b) A single parietal chloroplast is present.

c) Chlorella pyrenoidosa is often found in organically enriched waters. A dominance of Chlorella species is considered in some places to be an indication that a sewage stabilization pond is functioning to maximum capacity.

Scenedesmus

a) This genus is composed of a number of cells arranged with their long axis parallel to form a flat plate.

b) Scenedesmus forms a coenobial colony of up to 32, but usually 4 to 8 cells.

c) The appearance of cells of this genus may vary considerably with the species.

Pediastrum

a) Colonies (coenobial) of Pediastrum are free floating with up to 128 polygonal cells arranged in a single plane. There may or may not be spaces between the cells. The colonial shape of this genus usually resembles a gear-wheel.

b) The peripheral cells of each colony may differ in shape from the interior cells.

c) The exact arrangement of the cells of this genus seems to depend largely on the chance distribution of the original motile swarming zoospores at the time the coenobe was formed.

Cosmarium (desmid)

a) Cells of this genus are almost as wide as they are long and have a deep constriction across the center of the cell called the "isthmus" which forms two semi-cells.*

*NOTE: A distinctive group of green algae characterized by a median constriction dividing the cell into two bi-laterally similar halves is known generally as a "desmid", (Closterium and Penium do not have this constriction). Each half of the cell is known as a "semi-cell". The nucleus lies in the "isthmus" . Extremes of ornamentation and structural variety exist. Most are unicellular, but a few are filamentous or have the cells associated in shapeless colonies. They are found sparingly in the plankton almost everywhere but predominate in acid waters.

b) Cosmarium botrytis is reported in plankton from water supply reservoirs.

c) Some species have been reported to be sufficiently resistant to chlorine to penetrate rapid sand filters and may occur in distribution systems in considerable numbers.

Closterium - (desmid)

a) Cells of this genus are small in size; no median constriction is present. The cells taper slightly but are not sharply pointed.

b) Closterium cells have a slight crescent shape curvature in their long axis.

Schroederia

a) Members of this genus are solitary, and free floating. Individual cells are long and pointed at both ends and often form the shape of an "S".

b) In some species, spines may be present which protrude from the ends of the cells.

Selenastrum

a) Cells of this genus are pointed at both ends, and bent so that their tips approach each other.

b) Cells may occur in groups of 4-16. Also groups of cells may associate to form masses of a hundred or more cells.

Kirchneriella

a) Cells of this genus resemble Selenastrum but are much broader and bent into a definite "C".

b) Cells usually occur in groups of 4 to 8 in a broad, homogenous, gelatinous matrix.

Actinastrum

Actinastrum colonies are composed of 4, 8 or 16 elongate cells which radiate in all directions from a common centre.

Sphaerocystis

a) Colonies of Sphaerocystis are free floating and almost always with a perfectly spherical, homogeneous gelatinous envelope.

b) Up to 32 cells may be included in a single colony.

c) Sphaerocystis schroeteri is the only species recorded and is present in the plankton of lakes and reservoirs.

Coelastrum

a) Cells of this genus form coenobial colonies of up to 128 cells.

b) Both the cells and the colony are generally spherical in shape. Individual cells in the colony are connected by protoplasmic processes of varying length.

c) This genus may be differentiated from Sphaerocystis by not having a surrounding gelatinous envelope.

Micractinium

a) Cells of this genus are spherical to ellipsoidal and may be united in irregular 4-celled coenobial colonies.

b) The free surface of each cell in a colony bears from 1 to 7 slender hairs or setae.

Crucigenia

Cells of this genus forms free floating four-celled coenobial colonies. The colonies may be solitary or joined to one another to form plate-like coenobes of 16 or more cells.

Oocystis

a) The cells of Oocystis may be solitary, or up to 16 cells. Each cell or group of cells is surrounded by a partially gelatinized, expanded cell wall.

b) Individual cells are ellipsoidal to almost cylindrical.

Dimorphococcus

a) Cells of Dimorphococcus are arranged in groups of four. These tetrads are united to each other in irregularly shaped free floating colonies by the branching remains of the old mother-cell walls.

b) Two shapes of cells are normally found in each tetrad (the name suggests this). Two longer ovate cells end to end and a pair of shorter C-shaped cells on either side are present in each tetrad.

Staurastrum (desmid)

a) These desmids are radially symmetrical. Nearly all species have a deeply constricted isthmus.

b) Individual cells of this genus may be smooth, ornamented, or spined in a variety of ways.

c) Long truncated processes extend from the cell body.

Ankistrodesmus

a) Cells are long, slender and taper to a long point at each end.

b) Cells of this genus may be straight, curved, or twisted into loose aggregations.

c) Ankistrodesmus falcatus is often found in the plankton in water supplies and is considered to be one of the forms indicative of clean waters.

d) Single celled species called Monoraphidium in some texts.

LABORATORY - NON-MOTILE GREEN ALGAE

1. Using a taxonomic key, attempt to identify the alga provided by the instructor.

2. Draw a typical cell of Spirogyra using 450X, labelling all of the parts which make up the protoplasmic content of the cell.

3. Examine the slide preparations of green algae which are available, making sketches showing details of their structure for future reference. Sketch at least two coenobial types of green algae.

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FLAGELLATED ALGAE

1. Characteristics

a) All flagellates possess one or more flagella per cell. A flagellum is a whip-like appendage which acts as a propeller.

b) Some flagellates are unicellular, others are colonies of cells held together in gelatinous envelopes.

c) One or more chloroplasts are present in each cell. These chloroplasts contain chlorophyll.

d) Thick-walled resting stages may be assumed by certain species if unfavourable conditions are encountered.

e) Representatives of the pigmented flagellates may be found in several classes of algae.

2. Significance of Flagellates

a) Many pigmented flagellates can produce strong tastes and odours when they are present in water supplies. The flagellated alga Synura in small numbers can impart a perceptible cucumber odour to the raw water. Bacteriological doses of chlorine may aggravate the situation by changing the nature of the odour from cucumber to fish or oily.

b) Certain pigmented flagellates are able to withstand polluted conditions. These forms tend to indicate polluted conditions if other types which will not tolerate pollution are not present.

c) Large numbers of certain flagellates may cause filter-clogging problems.

d) Flagellates release oxygen to the water and utilize carbon dioxide.

3. Examples of Flagellated Algae

Pigmented flagellates may be found in the following classes Chlorophyceae (Greens), Euglenophyceae, Dinophyceae, Cryptophyceae and Chrysophyceae.

Chlorophyceae:

Chlamydomonas

a) Chlamydomonas is a solitary free-swimming genus. Cells with two flagella are usually round or oval in shape.

b) A single cup-shaped chloroplast is present.

c) Some species are characterized by a gelatinous sheath.

d) Members of Chlamydomonas are often found in oxidation ponds and polluted waters.

Carteria

This genus closely resembles Chlamydomonas but has four flagella instead of two.

Gonium

a) Gonium colonies typically have 4 to 32 cells arranged in a plate. The individual cells are embedded in a gelatinous matrix.

b) Sixteen-celled colonies move through the water in a somersault fashion while four and eight celled colonies swim flagella-end first.

Pandorina

a) Colonies of Pandorina range up to 32 cells. The colony is generally spherical.

b) The individual cells of this genus are arranged in a hollow sphere within a gelatinous matrix.

Eudorina

This spherical colony has up to 64 cells. The individual cells are deeply embedded in a gelatinous matrix. This genus is common in soft water lakes while Pandorina is more often encountered in hard water lakes.

Volvox

a) This colonial form rarely has less than 500 cells per colony.

b) The central portion of the mature colony may contain only water. Daughter colonies of Volvox form inside the parent colony.

Euglenophyceae:

Euglena

a) Cells of this genus are elongated, cylindrical or spindle-shaped and bear a single flagellum. Round forms of Euglena may occasionally be observed.

b) This form stores its food as paramylum (an insoluble carbohydrate) in numerous rod-shaped bodies.

c) A number of disc-shaped chloroplasts are present in the protoplasm of this genus.

d) Members of this genus are characterized by a red eyespot which is sensitive to variations in light intensity.

e) Because of the presence of the pigment haematochrome, some species of Euglena appear red in colour. With "bloom" conditions of Euglena sanguinea the entire surface of a pond will appear to be covered by a bright red film.

Phacus

a) Cells of this genus are often flattened and twisted, with a pointed tip or tail end.

b) The cell wall is marked with fine ridges.

c) This genus is characterized by one or more doughnut rings of paramylum.

d) Some forms such as Phacus pyrum are favoured by polluted water.

Trachelomonas

a) The protoplasm of this genus is enclosed in a brown shell called a "lorica" or "test" which may be oval or flask-shaped. This test has a hole or collar through which a single flagellum protrudes.

b) The surface of the test is usually brown in colour, and may appear smooth or rough.

c) Some species of this group have been known to clog filters.

Dinophyceae:

Ceratium

a) Ceratium is a member of the dinoflagellate group (armoured flagellate). This genus is distinctive in that one anterior and two posterior ends are continued as long horns. Members of this genus have a very distinctive form.

b) This genus is brown in colour.

c) Each cell of this group has a transverse groove and two flagella. One flagellum lies in the transverse groove.

d) Seasonal changes in temperature have a pronounced effect on the shape and number of these algal forms.

e) Ceratium hirundinella in high numbers has been reported to impart "vile stenches" to the water.

Peridinium

a) The cell walls of this group are thick, heavy, have a transverse groove and are usually highly ornamented.

b) Some members of this group may impart a fishy odour to the water.

Chrysophyceae:

Mallomonas

a) This is a solitary, free-swimming genus with one flagellum.

b) Each individual cell is covered with silicious plates, many of which bear long spines.

Synura

a) This is a colonial form.

b) Each pyriform-shaped cell has two flagella. The individual cells are radially arranged in the colony. Each cell may be characterized by the presence of two elongated, slightly bent chloroplasts.

c) Synura in very low numbers may impart a definite cucumber-like odour to the water.

Dinobryon

a) Cells of Dinobryon may be solitary, colonial, free-floating or attached.

b) Each cell is attached to the bottom of a lorica that has a closed, pointed base and an open, cylindrical or somewhat flaring apex.

c) Each cell of this genus has two flagella of unequal length.

d) This genus causes a fishy odour in the raw water.

LABORATORY - PIGMENTED FLAGELLATES

1. Examine some of the living flagellates in the samples provided, noting their corkscrew-like movement. Note how Euglena is able to change shape.

2. Follow through the key to identify Euglena. Make a sketch of this organism under medium power, slowing down the organisms with methocel so that the principal parts may be noted and sketched.

3. Examine the various prepared slides of the flagellates which are available and make sketches to demonstrate the features by which they may be identified.

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DIATOMS - BACILLARIOPHYCEAE

1. Characteristics

a) Diatoms have rigid cell walls which are made of silicon dioxide (silica or glass). The cells contain chromatophores and have a brown pigment in addition to chlorophyll. The individual cells or cell walls of this group are called frustules.

b) Diatoms may be unicellular, filamentous or colonial.

c) Each cell resembles a pillbox - two separate parts with one overlapping the other. The "valve" view is that of the top or bottom of the box. The "girdle" view is the side view.

d) Cells are either "pennate" which are elongated in structure or "centric" which affords one a circular view.

e) Diatoms reproduce mainly by cell division although formation of gametes do occur.

f) Cell markings are evident such as the "raphe" or pseudoraphe which extends longitudinally, and "striae" or "punctae" which are lines of pores extending from the raphe or pseudoraphe to the margin. "Nodules" at the ends of the raphe may also be present and internal shelves called "septa" are another feature.

g) Some diatoms can move slowly by a process called protoplasmic streaming.

2. Significance of Diatoms

a) Diatoms are the most important group of algae which cause filter-clogging. The most serious offenders are Asterionella, Fragilaria, Tabellaria and Synedra. The rigid silica wall of diatoms is not subject to decomposition. Therefore, even though the diatoms may die off rapidly on the surface of the filter, their silica walls remain to plug the pores in the sand.

b) Some diatoms such as Asterionella can produce tastes and odours in raw water supplies.

c) Water quality can be evaluated by specialists who understand the effects of polluted conditions on numbers of different diatoms.

3. Examples of Diatoms

Pennate Diatoms

Synedra

a) The frustules of this form are much longer than they are broad (symmetrical).

b) Synedra may occur as a solitary cell (most often) or in radially-arranged colonies.

c) The ends of each frustule are sometimes pointed, swollen or blunt.

d) A pseudoraphe is present.

e) Because Synedra is motile, it is capable of penetrating deeply into sand filter beds.

Fragilaria

a) These cells appear linear to fusiform in the valve view but are rectangular in the girdle view (symmetrical).

b) Cells of this genus unite to form a colony. The cells appear as a number of "cigars" stacked on top of each, other valve to valve.

c) A pseudoraphe is present.

Tabellaria

a) Cells of this genus unite in zigzag chains.

b) Each cell is long and slightly inflated at the centre and at each end.

c) A narrow pseudoraphe is present.

d) Longitudinal septa are present inside each cell of this group.

Navicula

a) Cells of this genus are symmetrical, elongate, and often boat-shaped. The cells appear rectangular in the girdle view.

b) Each cell has a raphe with central and polar nodules.

c) Members of this group are generally single-celled and free floating.

Nitzschia

a) Cells of this group are generally elongate as seen in the valve view. The sides of each cell may be constricted at the centre.

b) A true raphe is present.

c) On each valve, transverse striae or punctae are apparent.

d) Members of the genus Nitzschia are generally single-celled.

Asterionella

a) Individual cells are joined together in star-shaped colonies.

b) The inflated ends at the centre of the star are broader than at the free ends.

c) Cells of this genus should be compared to Tabellaria and Diatoma.

Surirella

a) Cells of this genus are elliptical or oval-shaped with rounded ends (asymmetrical).

b) Surirella occurs as relatively large elongated elliptical cells with heavy transverse costae.

Meridion

a) Individual cells of this genus are wedge-shaped in girdle view and form fan-shaped colonies which are joined valve to valve.

b) Members of this group have internal septa which show through the wall of the frustule.

Cocconeis

Frustules of this diatom are broadly elliptical in the valve view and transversely curved in the girdle view. The two valves are similar in outline but dissimilar in structure.

Pinnularia

a) The symmetrical frustules of Pinnularia have valves that are usually with rounded poles and straight parallel sides.

b) Frustules of Pinnularia are usually solitary and free floating.

Gomphonema

a) This genus is distinguished from other naviculoid diatoms by having frustules that are transversely asymmetrical in both valve and girdle views.

b) The valves are straight with one pole broader than the other.

c) Frustules of Gomphonema are usually epiphytic and present on filamentous algal forms. Sometimes the frustules are sessile.

Centric Diatoms

Cyclotella

a) Cells of this genus are circular in the valve view. A smooth region in the centre and a peripheral lined region characterizes the valve view of this form.

b) Cyclotella is generally solitary and free floating.

Stephanodiscus

a) Cells are circular in the valve view.

b) The rows of puncta on the surface of the valve extend into the centre of the cell.

c) Some species of this genus are characterized by the presence of spines which extend from the wall of the frustule.

Melosira

a) Cells of Melosira are cylindrical, sometimes with convex valves.

b) Some species such as Melosira granulata have terminal cells which have robust spines. These spines or teeth assist in holding the individual cells together in a filament. These filaments can be quite long.

c) Melosira at low magnification appears similar to the filamentous green alga Ulothrix.

LABORATORY - DIATOMS

1. Using some of the micro-strainer waste which is available, make up a slide and observe under low power.

2. Note the varied forms which are present, their colour etc., and by pressing lightly on the coverslip, attempt to change the position of individual forms so that different views of the cells may be presented.

3. Examine the prepared diatom mounts and identify as many genera as you are able.

4. Note the presence of a true raphe in Navicula and a pseudo-raphe (false raphe) in Synedra. The pseudoraphe is formed by interruptions in the lines of dots (punctae) which run transversely across the cell.

5. Make drawings of Stephanodiscus and Navicula under medium power, identifying and naming as many of the structural features as possible.

6. Study and make sketches of as many diatoms as possible, using the reference material available to identify the different genera, with the help of the instructors present.

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WATER SUPPLY SAMPLING INVESTIGATIONS

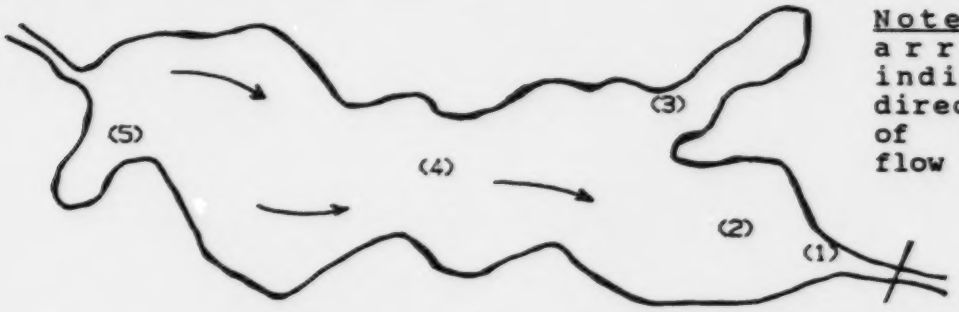
1. An organized program of plankton counting should be based on samples taken at least once a week.

2. Seasonal patterns of algae development tend to repeat themselves year after year and so are relatively predictable - a certain amount of seasonal variation is to be expected, (variables such as nutrients, rainfall, temperature).

3. More analyses may be desirable if population of a troublesome species increases.

4. It may be easier and less costly to control an upsurge of one particular alga in its early stages.

Location of Sampling Points



Note : arrows indicate direction of water flow

a) Both shallow and deep samples should be taken.

b) Samples from top to bottom at one sampling point may be composited to provide a summary for that station.

c) Each major bay or shoal area of a reservoir should be sampled and one should be taken near an intake if the latter is present.

d) Sampling over 24-hour periods or longer may be helpful either studied individually or composited.

e) Samples may be taken from the intake pipe, at the low lift well, or from a "raw water" tap in the plant laboratory, provided that it is flowing continuously.

Preserving Samples

a) Samples submitted to the laboratory must be preserved.

b) Lugol's solution is most commonly used. It is a mixture of potassium iodide (100 g) and iodine crystals (50 g), glacial acetic acid (95 mL) dissolved in 1000 mL distilled water. The amount required to preserve a sample is that amount which will give the sample a deep amber (tea) colour - usually 2 mL. Lugol's solution per 1 litre sample.

c) A minimum of preservative may be used to render organisms immobile without altering the physiology of the cell too much.

d) Because some flagellated forms are delicate and destroyed when a preservative is added, it may be desirable to obtain a duplicate sample, one preserved and the other unpreserved (live) from the same sampling location.

e) Formalin at 3-5% causes shrinkage of cells and can cause flagellates to explode if these are in the sample.

f) A .1% merthiolate solution will adequately preserve plankton up to six weeks.

CONCENTRATION TECHNIQUE FOR ALGAE ENUMERATION

Due to several factors such as too few organisms per field, occasional large organisms in isolated fields, clumping, etc., unsatisfactory algae counts can be obtained. To increase the accuracy of algae counts, it is essential to employ a concentration technique by which a large sample volume is reduced to a few millilitres. It is then possible to enumerate organisms in a relatively small number of fields rather than covering an entire strip or perhaps half the cell volume. An appropriate concentration factor must be inserted in the formula to calculate the quantity of algae present per mL of sample.

METHODS AND MATERIALS

Day 1: a) Use a 1 litre glass container to collect a 1000 mL (approx.) sample of raw water each week from the Microstrainer inlet channel, the raw water intake well or from the raw water tap in the laboratory. (This tap should be continuously flowing).

b) Add 2 mL of Lugol's solution to this 1000 mL and shake gently.

c) During the months of December, January, February and March use a 500 mL graduated cylinder and fill to 500 mL with the preserved raw water sample. During the months of April through to the end of November, use a 250 mL graduated cylinder and fill with 250 mL of preserved raw water sample.

d) Add 5 drops of Lugol's solution to the graduated cylinder and let stand.

Day 2: Add 5 drops of Lugol's solution to the sample in the graduated cylinder and let stand.

Day 3: a) Without agitating the graduated cylinder, use the tap aspirator, a rubber hose and pipet and siphon off the supernatant water down to the 80 mL mark of the graduated cylinder. Take care to keep the tip of the pipet just below the water surface as sample is siphoned down.

b) Shake the remaining 80 mL of sample and transfer from the 500 (or 250 mL) graduated cylinder to a 100 mL cylinder.

c) Using a 25 mL graduate measure out 20 mL of distilled H_2O or tap water. Use this to rinse out the 500 mL (250 mL) cylinder and then pour this rinse water into the 100 mL cylinder.

d) Add 5 drops of Lugol's solution and let stand.

Day 4:

a) Without agitating the 100 mL graduated cylinder, use the tap aspirator to siphon off the supernatant water down to the 20 mL mark. Take care to keep the tip of the pipet just below the water surface and do not go below the 20 mL mark.

b) Shake the remaining 20 mL of sample (which should now contain all the algae from the original sample) and transfer it to a 1 oz (25 mL) vial. Rinse the 25 mL graduate with 5 mL of distilled H_2O. Use a 5 mL pipet for this purpose and transfer this 5 mL of rinse water into the 1 oz vial.

c) Add 1 or 2 drops of Lugol's solution to the vial if the colour has faded.

d) Cap tightly and label with date, number, plant, location and concentration. The concentration should be marked on the bottle

like this	500 -> 25
or	250 -> 25
	100 -> 25

This way we know your original volume and final volume.

500 to 25 means it has been concentrated 20 times (20X)
250 to 25 means it has been concentrated 10 times (10X)
100 to 25 means it has been concentrated 4 times (4X)

CALCULATION

If a 500 mL sample is reduced to 20 mL + 5 mL of distilled wash water, then the concentration factor is:

$$\frac{500}{25} = 20X$$

One mL of the concentrated sample is placed in a Sedgewick-Rafter cell for enumeration. Five to twenty-five fields or up to two strips or more may be counted depending on the density of the organisms present. If counting by fields, it is considered that a degree of concentration that will provide no less than 10-20 organisms per field is essential to ensure reasonable statistical accuracy.

In determining the total count, the following formula is used:

$$\frac{\text{total square microns X Factor (varies with fields counted)}}{\text{400 X concentration factor}}$$

The concentration technique should not be used if the sample contains high levels of algae (especially filamentous forms which tend to clump) or large quantities of organic debris.

The Sedgewick-Rafter Concentration technique is not commonly used anymore. The Sedgewick-Rafter funnel, sand and silk bolting cloth all contributed to losses and breakage of delicate algal forms. Centrifuging or membrane filtration techniques cause similar problems. Straight sedimentation using Lugol's Iodine increases the specific gravity of the organisms to aid in settling, stains the starch bodies (pyrenoids) and preserves the organism all in one effort.

PROCEDURE FOR POOLING SAMPLES FOR "AVERAGE ANALYSIS" METHODS

A number of samples are collected on a regular weekly basis throughout the sampling season. However, the total samples collected from any one location may be recombined into one composited sample and counted as a single analysis from that location at the end of the month or season. In order to recombine the samples accurately and to maintain the correct concentration in the final aliquot the procedures outlined must be followed carefully. The two examples below show how to calculate the concentration factor to be used for the calculation of the recombined sample.

Example 1

Consider Lake 'A' with 5 samples (e.g. 1 per week in July), these samples are submitted weekly and concentrated as indicated in column 2 below - (initial concentration). The procedure for combining these samples for a single "average" count and the calculation of the final concentration factor for the pooled sample is given below. Note that the lake equivalent volume (column 4) of each subsample must be the same within any set of samples, i.e. (column 2) x (column 3) = (column 4).

Lake A - Recombined for Monthly Mean Composite

(1) Sample No. or Date	(2) initial conc.	(3) subsample volume	=	(4) lake equivalent volume
July 1	4X	1 mL	=	4 mL
" 8	4X	1 mL	=	4 mL
" 15	4X	1 mL	=	4 mL
" 22	4X	1 mL	=	4 mL
" 29	1X - unconc.	4 mL	=	4 mL
		8 mL		20 mL

All algae are now in 8 mL of water (new combined sample) that were originally in total of 20 mL lake water. Therefore, the concentration factor for the recombined (pooled) sample is the quotient obtained from the sum of column (4) divided by the sum of column (3):

$$\text{i.e. } \frac{20 \text{ mL}}{8 \text{ mL}} = [2.50]$$

Lake B - Recombined for Summer Seasonal Composite (11 weeks)

(1) Sample No. or Date	(2) initial conc.	x (3) subsample volume	= (4) lake equivalent volume
1	10X	1 mL	= 10 mL
2	10X	1 mL	= 10 mL
3	10X	1 mL	= 10 mL
4	4X	2.5 mL	= 10 mL
5	4X	2.5 mL	= 10 mL
6	10X	1 mL	= 10 mL
7	4X	2.5 mL	= 10 mL
8	1X unconc.	10 mL	= 10 mL
9	1X unconc.	10 mL	= 10 mL
10	4X	2.5 mL	= 10 mL
11	10X	1 mL	= 10 mL
		35 mL	110 mL

All algae are now in 35 mL of water (new combined sample) that were originally in total of 110 mL lake water. Therefore, the concentration factor for the recombined sample is:

i.e. $\frac{110\ mL}{35\ mL}$. [3.14]

A similar table should be prepared for each set of lake samples handled in this way and attached to the bench sheet for the recombined sample.

Detailed Procedures

1) Using a regular bench sheet, prepare a table as in examples above for each set of samples. List sample number, date, initial concentration, subsample volume and lake equivalent. Calculate and record the concentration factor for the recombined sample (to 2 decimal places).

2) Select appropriate glassware and pipets for measuring aliquots of each sample. We now have 1, 5 and 10 mL disposable serological pipets. The 5 mL disposable serological pipets are for measuring quantities from 1.1 to 5 mL. Serological pipets are designed to deliver to the tip which means the small amount remaining in the tip after free delivery must be blown out and added to the main volume delivered. Care should also be taken to remove any drops that adhere to the outside of the pipet as you take it out of the original container. These

drops should be returned to the original container by touching the pipet against the inner edge of the container as it (the pipet) is withdrawn from the container.

3) The individual aliquots of subsample should be placed in an appropriately sized graduated cylinder for mixing of the recombined sample (i.e. 50 mL, 125 mL or 250 mL flask). The recombined sample should not more than half fill the Erlenmeyer flask in which it is contained. After thoroughly mixing by gentle agitation (a parafilm cap can be used to allow inversion of the flask for thorough agitation) and/or by blowing air through a pipet into the sample, a 1 mL aliquot can be removed for placement into a counting chamber. Retain the recombined sample in the graduated cylinder.

4) Examine the aliquot of sample in the counting chamber to determine if the distribution of organisms is adequate for counting. If the distribution is too dense, take a smaller aliquot (i.e. 0.5 mL). If the distribution is too sparse take a larger aliquot (2 to 5 mL) but remember that any change from a 1 mL aliquot will alter the concentration factor and should be noted.

5) The remaining portion of sample in the graduated cylinder should again be thoroughly mixed and all/or an aliquot of this sample should then be placed in a 25 mL (1 oz.) vial and re-entered into our submission book as a new sample number.

6) The new recombined sample number should be used on the bench sheet, the analysis card and the bench sheet which was used for calculating the concentration factor. The bench sheet used for counting the sample should have the word "re-combined" concentration factor entered. As mentioned in item 4) above, any aliquot other than 1 mL should be entered here as a product of the concentration factor (i.e. recombined conc. factor = 2.5 x 0.5 or 2.5 x 1 etc.).

7) When completing the Plankton Analysis Card the following information should be included:

Date sampled:	First day to last day (i.e. May 5 - Oct. 10, 1988)
Depth:	Photic zone composite
Enumeration procedure:	Recombined - 1 strip etc.
Concentration factor:	Recombined - 2.50
Total units:	Total Cubic Micrometers per mL or Total Areal Standard Unit per mL
Composite total:	Seasonal average or Monthly Mean
Remarks:	Composite of 5 samples

USE OF THE MICROSCOPE FOR ALGAE ENUMERATION

It is assumed that persons using this manual are familiar with the use of the microscope. However, some basic procedures are worth repeating. Use the diagram on page 4-2 and 12-3 to identify the parts of the microscope in this section.

A compound microscope should be used having the following features:

i) a good light source with a variable transformer preferably built into the base with Koehler illumination.

ii) a rectangular mechanical stage with graduations and coaxial movements.

iii) an objective turret containing 4X, 10X, 20X and 40X objectives (parafocal achromatic or planachromats).

iv) Binocular head containing 10X widefield ocular lenses one of which should be capable of holding a Whipple ocular grid (see diagram P. 12-4) and with an adjustable focusing collar.

To use the microscope properly you must be able to turn on the light source, align the light path properly, select a low power (10X) objective and place a specimen on the stage for observation. Observing the specimen on the stage by looking at the side of the microscope use the coaxial adjustments to put the specimen into the centre of the stage and intercept the light path.

Then, continuing to look at the side of the microscope raise the stage so that the specimen slide is very close to the 10X objective. Noting which direction to turn the stage away from you, look into the eyepiece and lower the stage with the course focus adjustment until the specimen image comes into focus. Then use the fine focus adjustment to sharpen the image. At this point, you may rotate the objective turret around to swing the 20X objective into place. If you are using parafocal objectives, you should be able to bring the specimen into focus again by using the fine focus control.

Remember to:

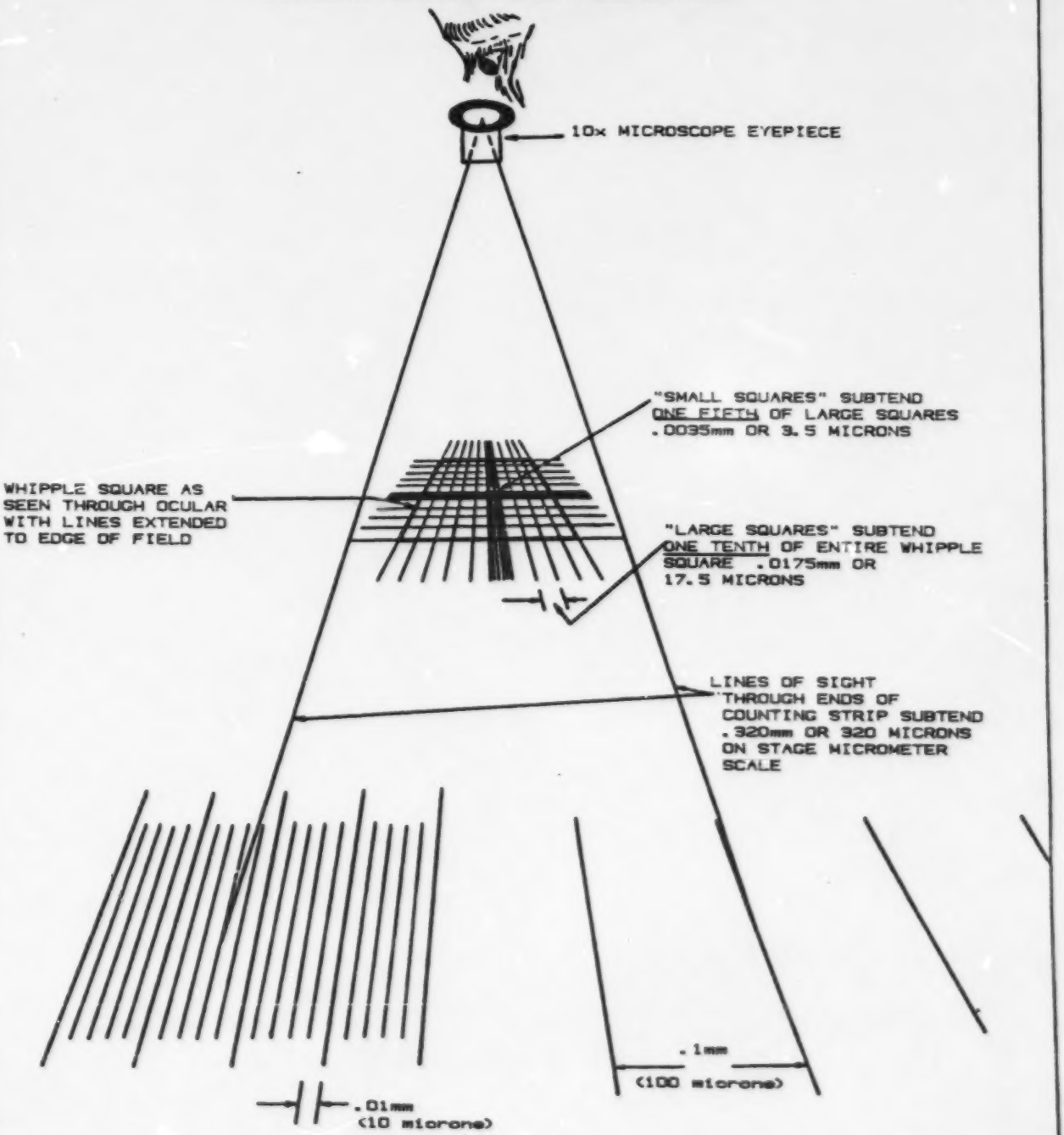
1) Always start with the low power objective and work up to the next higher one.

2) If you are using a Sedgewick-Rafter counting chamber to hold your specimen, you will be limited to the 10X and 20X objective for observations.

3) Always watch from the side of the microscope if you have to raise the stage to find the specimen.

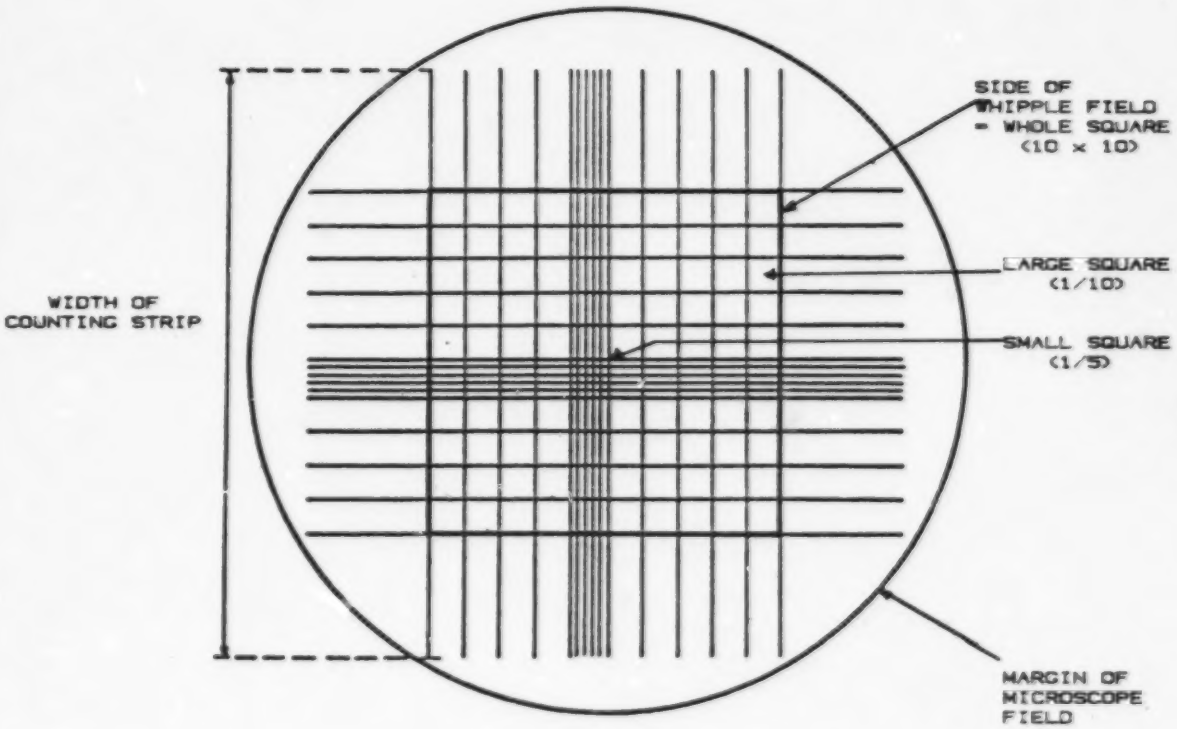
4) Always move the stage away from the objective lens to bring the specimen into focus.

DIAGRAMATIC CALIBRATION OF WHIPPLE SQUARE
FOR MOE MICROSCOPE
NO. 1130
AS SEEN WITH 10x OCULAR AND 40x OBJECTIVE
(APPROXIMATELY 400x TOTAL MAGNIFICATION)


10x MICROSCOPE EYEPIECE
"SMALL SQUARES" SUBTEND ONE FIFTH OF LARGE SQUARES .0035mm OR 3.5 MICRONS
WHIPPLE SQUARE AS SEEN THROUGH OCULAR WITH LINES EXTENDED TO EDGE OF FIELD
"LARGE SQUARES" SUBTEND ONE TENTH OF ENTIRE WHIPPLE SQUARE .0175mm OR 17.5 MICRONS
LINES OF SIGHT THROUGH ENDS OF COUNTING STRIP SUBTEND .320mm OR 320 MICRONS ON STAGE MICROMETER SCALE
.1mm
(100 microns)
.01mm
(10 microns)

PORTION OF MAGNIFIED IMAGE OF STAGE MICROMETER SCALE

WHIPPLE COUNTING GRID
AS SEEN THROUGH MICROSCOPE EYEPIECE


SIDE OF WHIPPLE FIELD = WHOLE SQUARE (10 x 10)
LARGE SQUARE (1/10)
SMALL SQUARE (1/5)
WIDTH OF COUNTING STRIP
MARGIN OF MICROSCOPE FIELD

MICROSCOPE CALIBRATION DATA

* 1MM = 1000 MICROMETERS

COMPOUND MICROSCOPE NO. _____

		Linear Dimensions of Whipple Square in Millimeters *			Width of Entire Field	Factor for Conversion to Count/mL
	OBJECTIVE	WHOLE	LARGE	SMALL		(1 S-R STRIP)
OCULAR	10x					
10x	20x					
	40x					
	100x					

INVERTED MICROSCOPE NO. _____

	OBJECTIVE	WHOLE	LARGE	SMALL		(1 RADIUS) Utermohl Chamber
OCULAR	10x					
10x	20x					
	40x					
	100x					

LABORATORY - QUALITATIVE EXAMINATION OF MIXED PLANKTON

1. Using the sample of raw water provided, study and identify the algae present and attempt to list them under the four major groups of algae which have been previously studied in detail. Use the texts available to help in your identifications and solicit the assistance of the instructors present.

2. If you would like additional practice with any one of the major groups ask the instructor to provide the necessary cultures.

3. Use the key to help sort the algae into proper groups. If you do not understand the terminology always check the glossary of terms.

CALIBRATION OF PLANKTON COUNTING EQUIPMENT

A Whipple Plankton Counting grid must be used in microscopes to delineate the width of counting strips and for measuring areal value of individual organisms. Since the optics of no two microscopes are exactly the same, it is necessary to "calibrate" each instrument against a known scale to determine the linear values of the lines or areal values of the squares making up the counting grid. The Whipple eyepiece grid must be calibrated for each magnification that is to be used. Formerly microscopes having an adjustable tube length could be set so that the square which was part of the grid covered an area of one square millimetre on the counting cell. This occurred when the tube length was 160 mm. However, most modern models of microscopes do not have an adjustable tube length.

1. Procedures Involved in Calibration

a) Installation

To install the ocular micrometer in the eyepiece carefully unscrew the upper lens and insert the disc, allowing it to slide down until it comes to rest on the shelf inside. Replace the lens and look into it. If the markings on the counting grid are not in sharp focus, remove the disc and turn it over.

b) Lenses Normally Used in Counting Plankton

The 10X eyepiece and the 20X objective are normally used in counting plankton, to provide a total magnification of 200X. The 40X and 100X objective cannot be used with the S-R cell because of the short working distance beneath these medium power objectives.

c) The Stage Micrometer

The stage micrometer is actually a tiny ruler which is placed on the stage to measure the dimensions of the lines making up the counting grid. The lengths of the various segments of the lines making up the grid should be determined and recorded on the Calibration Data Sheet which is presented on page 12-5. This must be repeated for each person and for each combination of lenses employed.(see completed calibration charts p.20-1).

d) Calibrating the Microscope with the Stage Micrometer

Using the stage micrometer ruler as your specimen slide, place it on the stage and move it with the coaxial adjustments so that the circled ruler is at the centre. Swing the 10X objective into place and looking from the side of the microscope raise the stage close to the

micrometer slide. Looking through the eyepiece, move the stage away from you by turning the course focus adjustment until the image of the stage micrometer ruler comes into focus. Use the fine focus control to sharpen the image.(see diagrams p.12-3 and p.12-4)

The stage micrometer ruler will have a zero line on the left and course and fine divisions marked at 0.01 mm and numbered ever 0.1 mm for a total distance of 2.0 mm. One millimetre is equal to 1000 microns, therefore each division of the ruler is 10 microns.

Orient the ocular grid (the Whipple grid) so that the grid lines appears vertical and parallel to the vertical stage micrometer lines. Move the stage micrometer using the coaxial stage movement control knobs so that the zero line of the ruler is superimposed on the last vertical line on the left of the Whipple grid. Now look to the last vertical line on the right of the Whipple grid (i.e. the tenth one) and read off the number of ruler divisions and record this measurement in the table on page 12-5 as the measurement for the width of the WHOLE square. Now measure the distance between the first and second vertical line from the left as the measurement of the LARGE square. The WHOLE square is equal to 10 LARGE squares.

Now move the zero line of the stage micrometer so that it falls on the left vertical line of the fine divisions at the centre of the Whipple grid. As there are five divisions at the centre, the measurement of five SMALL squares is equal to one LARGE square. With a 10X ocular and a 10X objective it may not be possible to accurately measure one SMALL square.

Now place the zero line of the stage micrometer on the left end of the horizontal lines of the Whipple grid and read the measurement on the right end of the horizontal lines. This measurement will give you the total viewing WIDTH of the ocular field covered by the Whipple grid.

Now change to the next higher objective (20X) and repeat these procedures to get the measurements of the WHOLE square, LARGE square, SMALL square and the WIDTH of the entire field.

Now change to the 40X objective and repeat these procedures again. It may not be possible to accurately measure the distance between the SMALL squares as the ruler lines are much thicker now than the ocular grid lines.

By examining the figures in the table created on page 20-1 it may be seen that as the magnification increases from 100X to 200X that the size of the squares and the width

of the field decrease by one-half (approximately).

Use the bottom half of the chart to convert these measurements into micrometer units by moving the decimal 3 places to the right. (i.e. 0.620 mm = 620 micrometers and 0.035 mm = 35 micrometers). Micrometer replaces micron and is written in symbolized form as μm (i.e. 14μm).

2. **Calculating Factors to Convert to Counts per mL**

a) **Strip Counts**

The S-R cell is 50 mm long x 20 mm wide by 1 mm deep. The total volume of the cell is therefore 1000 mm^3 or 1 mL. Factor for one STRIP - One strip is delineated by the distance between the ends of the vertical lines of the Whipple grid and is equal to the measurement in the table on page 20-1 under the column "Width of Entire Field". Starting at one side of the Sedgewick-Rafter counting chamber and using the coaxial controls move the chamber across the stage and note all the algal organisms that fall between the ends of the vertical lines (width of the strip) all the way to the other end of the chamber. This is in fact a distance of 50 mm.

Volume = length x width* x depth

= 50 x .5* x 1

= 25 mm^3

*(this measurement varies with each microscope and each microscopist)

It is necessary to multiply the total count for each genus observed in one strip by a factor obtained by dividing the volume of the strip into the total volume of the cell.

Using our example, the Factor for 1 strip = $\frac{1000\ mm^3}{25\ mm^3} = 40$

The counting chamber would contain 40 strips at .5 mm width.

If two strips are counted, then of course the multiplier factor would be cut in half - i.e. 20.

If one-half strip is counted, then the multiplier factor is doubled - i.e. 80. See the table on page 16-10 for a typical set of factors for 1/4 to 5 strips.

b) Field Counts

If high numbers of algae are present in a sample it might not be necessary to count one full strip, although this is likely to be the exception rather than the rule.

When high numbers are encountered, 10, 20 or 40 fields may be examined and each field is delimited by the four lines making up a WHOLE square in the ocular grid. (Page 12-4). The basic relationship previously described still applies, as follows:

$$\text{Factor} = \frac{\text{volume of cell in mm}^3}{\text{volume examined in mm}^3}$$

The total volume of the area examined using 10 fields is determined by multiplying the total area of the 10 fields by the depth. Therefore, when the sides of the WHOLE square (i.e. one WHOLE field) in the ocular grid represent, for example, a length of .3 mm (or 300 micrometers) the volume for 10 fields is as follows:

Volume = (side of Whipple field)^2 x depth (1 mm) x (# of fields counted)

$= .3^* \times .3 \times 1 \times 10$

$= .9$ mm

*(this measurement varies with each microscope and each microscopist)

Therefore the Factor for 10 fields

$$= \frac{\text{Volume of cell in mm}^3}{\text{Volume examined in mm}^3}$$

$$= \frac{1000 \text{ mm}^3}{.9 \text{ mm}^3}$$

$$= 1111$$

The counting chamber would contain 1111 field at .3 mm X .3 mm width.

If 20 fields were counted, the factor would then become 555.

Note: The fields should be selected at random by dividing the chamber into 10, 20 or 40 sections.

To convert the Total Square Microns measured to Areal Standard Units per millilitre (A.S.U./mL)

$$\frac{\text{Total Sq. Microns X Microscope Factor (F)}}{400 \quad \text{X Concent. factor (f)}} = \text{A.S.U./mL}$$

1 Areal Standard Unit is the Area Subtended by 400 square microns.

See Table of Combined Factors on page 16-10 for different fields/strips counted at different concentrations. This table is specific for each microscope and each analyst. A separate table should be prepared for each individual.

ENUMERATION OF PLANKTON

PREPARATION

1. Whenever possible samples should be studied initially without a preservative being added. Certain of the flagellate forms will either disappear entirely or be severely distorted by formalin as low as 1%. Lugol's Iodine preservative may be added to kill motile forms for counting after their identity has been established.

2. Unpreserved samples may be refrigerated for future analysis but if they are to be held more than a couple of days, preservative should be added.

3. Samples may be concentrated as outlined in a previous section to obtain sufficient material for examination or the sample may be pooled with other samples to observe the seasonal composition of algal growth.

ENUMERATION

Qualitative and Quantitative Analyses

It may only be necessary at times to determine what types of plankton are present and to obtain an approximate idea of their relative numbers. Perhaps a taste and odour problem has developed and it is only essential to determine what particular alga is causing the problem.

However, for analyses to be of lasting value, they must provide a relatively accurate measurement of the numbers of plankton for each genus present. If annual records are maintained, predictions may be ventured since similar conditions tend to develop in cyclic fashion year after year. However, seasonal variations do occur because of factors such as water temperature and amount of sunshine. Information related to water temperature, nature of the weather and cloud cover, water turbidity and pH is all valuable and may lead to more knowledgeable interpretations of what has happened in the past and what may be expected in the future. It is only after records have been maintained for several years that accurate forecasting may be a certain accomplishment.

Regular quantitative counts should be made weekly at least. This should provide adequate forewarning of the increase of one particular alga to nuisance proportions so that remedial measures may be implemented before the nuisance condition is an established fact.

Standardized Techniques for Quantitative Counts

The "clump count" has been used in the past but the major disadvantage of this simpler method is that filaments and colonies

are counted as units, equal to individual cells. No cognizance is taken of the relative masses of the various organisms which are present.

Two standardized techniques are used in our laboratories for the quantitative measurement of algae. Areal Counts reported in Areal Standard Units are used for water supplies and Volumetric Counts are used for other technical studies where the biomass or biovolume of algae is required to relate it to other chemical and physical parameters.

Areal measurements (surface area) of most algal cells can be made in a 1 mL Sedgewick-Rafter chamber but volumetric measurements (length, width and depth) particularly of smaller algal cells require higher magnifications not attainable in a S-R cell. Therefore, we use Utermöhl chambers and inverted microscopes which allow observations to be made using high power objectives.

Other methods of quantitative measurement include counting cell numbers per mL, measuring Chlorophyll a, dry weight of algal material, or adenosine triphosphate (ATP) as described in Standard Methods (APHA).

AREAL COUNTS

Procedures related to the areal standard unit (A.S.U.) method of count used by waterworks personnel are as follows:

1. a) Sedgewick-Rafter counting cells which measure 50 mm X 20 mm X 1 mm holding exactly 1 mL of sample are used. They are provided with special thin cover glasses.

   b) The S-R cell is filled by drawing slightly in excess of 1 mL of the sample into a pipette after the sample has been well shaken and allowing the sample to flow into one of the openings created by laying a cover glass diagonally across the counting cell. If this is done properly the air will escape from the opening on the opposite side and the cover glass will rotate into proper position by itself to cover the cell. Excess water can be removed by wiping lightly with a soft tissue.

   c) After filling the S-R cell, it should be allowed to sit on the microscope stage for several minutes to allow the organisms to settle out.

2. The areal values of algae in one or two strips across the cell are recorded, or perhaps the areal values for organisms present in a predetermined number of fields, (for a concentrated sample) and recorded. Using appropriate multiplier factors these areal values are projected to areal standard units per mL, litre, etc. An areal standard unit is the area subtended by 400 square microns.

3. The area which needs to be examined varies with the concentration of algae in the sample. Generally, ten fields are enumerated in a concentrated sample. If the sample is not concentrated because of high numbers of algae, one, two or more strips may need to be examined.

4. Half of the cell should be checked using low (10X) power to determine whether rotifers or other animal plankton are present in the sample. In this "survey count" the actual numbers of organisms are recorded instead of their areal values.

5. Before areal standard unit counts can be attempted, the microscope must be calibrated so that the linear and areal values of the lines and squares associated with the ocular micrometer are known (outlined in section on Calibration). Microscopic counts using the Sedgewick-Rafter counting chamber are limited to 200X magnification due to the limited working distance of the 20X objective.

6. Before starting the count the sample should be scanned to determine what organisms are present and to establish average areal values for types of algae which are abundant and relatively consistent in size. It may be necessary to use the medium power objective to determine the areal value of individual organisms more accurately. If this is the case, an ordinary slide must be used and it might be necessary to use a concentrated sample for this purpose.

7. Measurements of individual cells, filaments or colonies can be achieved by manipulating the organism into the centre of the field of view and superimposing the edge of a line of the ocular grid over the edge of the cell to be measured. For long filaments or rectangular colonies it may be necessary to rotate the ocular grid until it is parallel to the organism to be measured. If the width of the organism does not equal the width of a fine division of the ocular grid it may be necessary to estimate the final portion. (i.e. the cell width may be 2 1/3rd small divisions). Through practice these measuring manipulations will become much easier.

8. Records should be kept of the average areal values of individual species to avoid needless repetition of preliminary measurements. Different species of the same genera may be recorded as Species A, B, C, etc., with their distinguishing features being noted.

9. For the first while it will be necessary to measure each cell, filament or colony which is encountered to determine its areal value. The individual areal values assigned for each genus are recorded on the bench sheet (p. 20-2). When the count is completed, the total areal value for each genus is obtained, and multiplied by the <u>appropriate</u> factors to obtain the number of areal standard units per millilitre (A.S.U./mL).

10. See page 15-7 for procedures and formulae to use when measuring the surface area of geometric shapes.

VOLUME COUNTS

Volumetric Counts, in which the third dimension (length, width, depth) of an organism is used to determine the true biomass or biovolume of algae per volume of water. Volumetric counts are normally performed using inverted microscopes and Utermöhl sedimentation chambers (2, 5 or 10 mL). This allows specimens to be examined using high dry or oil immersion objective lenses. Due to the restrictions imposed by the use of a normal compound microscope and the S-R counting cell this method is not commonly used by waterworks personnel. It is described here for the benefit of others involved in other quantitative plankton studies.

1. Setting up Samples for Volumetric Counts

Samples preserved for volumetric phytoplankton enumeration are stored in 25 mL vials. Some have been concentrated and all are preserved with Lugol's solution and formalin. Each vial should be shaken and the amount of sample to be examined should be quickly pipeted out and blown into a 10 mL beaker. The amount to be examined is usually 1 mL so 1 mL pipets are used.

If 1 mL is to be examined, add 4 mL of distilled water to the sample already in the beaker. Thorough mixing of the sample and the water is accomplished by bubbling with the pipet and stirring simultaneously.

This 5 mL sample is then poured quickly into a 5 mL Utermöhl chamber and the cover glass is then slid transversely across the chamber top to seal the sample in the chamber. Cover glasses that are scratched or chipped cause bubbles to form in the chamber after a short period of time.

If you find that the settled sample (i.e. allowed to stand at least 4 hours) is not dense enough to count (i.e. less than 50 pieces in 1 radius) you should resettle the sample using from 2 to 5 mL of sample and the corresponding amount of distilled water to make it up to 5 mL. You must also adjust the concentration factor accordingly. If the sample is too thick you can take a minimum of 0.5 mL sample and 4.5 mL water.

2. Standardization of Counting Procedures for Areal and Volumetric Counts

Volumetric samples are normally counted using the 40X objective, however, large biomass individuals should be counted at a lower magnification so that all inverted microscopes should be calibrated for the 20X objective. If, when you scan the chamber, you notice that certain large

pieces of algae (i.e. Ceratium) are unevenly or sparsely distributed it would be advisable to count the entire chamber bottom at a lower magnification. This should be done even if you did not get a large biomass occurring in your counted area.

A minimum of 300 pieces, meaning singly occurring cells or colonies, should be counted. If you have colonial forms such as Asterionella or Fragilaria make sure that you differentiate the colonies from the individual cells (i.e. Asterionella formosa (75 x 2^d) 8.8.8, means 3 colonies of 8 cells each at 75μ long by 2μ diameter). Colonies are indicated with a number, whereas, single cells are indicated with a stroke. One colony counts as one piece. In large cells such as Ceratium where you have to do several measurements, enclose one cell's measurements in square parentheses i.e. [(10 x 20 x 7) (8 x 15 x 7) (30 x 5^d)]. See Sample Bench Sheet p.20-5.

3. Check for E_{max}

If you have a large number of one genus i.e. chrysomonads, count only 1 radius or approximately 50 individuals and continue on to count more of the less numerous pieces. Once the count is completed, calculate the total biomass and determine the 6-10 dominant species or genera that make up approximately 90% of the biomass, then test for the $Error_{max}$.

($E_{max} = \pm 2 \frac{100\%}{\sqrt{N}}$) of these genera to determine if your count meets the following guidelines:

a) if a single genus is 50% of total biomass count 45-100 units to achieve an E_{max} of 20-30%, or

b) if a single genus is >20% but <50% of total biomass count 30-50 units to achieve an E_{max} of 28-37%, or

c) if one genus is between 10-20% of total biomass count 25-30 units to achieve an E_{max} of 37-40%, or

d) combination of a, b, and c.

Counts of less than 10-20 for an individual taxon are very unreliable.

e.g. $E_{max} = \pm 2 \frac{100\%}{\sqrt{N}}$ where N = 10

$$E_{max} = \frac{200}{\sqrt{N}} = 63\%$$

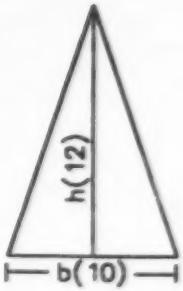
4. Bench Sheet Records

On the bench sheets, we would like to standardize the method of recording measurement so that we can quickly determine how the measurements were made and how the total biomass was derived.

The sample bench sheet on P. 20-2 and P. 20-6 show you how to set up and calculate a sample. Note that only 1 combined factor should be used in spite of 3 different enumeration procedures. Also note the decimal places used for Microscope Factor (F), (floating point); the "Totals" Column (2 places); the concentration factor (f), (2 places); the combined factor (c), (floating point).

5. Procedure and Formulae for Measuring the "SURFACE AREA" of Geometric Shapes

TRIANGLE



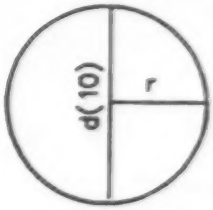
b = base h = height

area = $\frac{b \times h}{2}$

This should be designated on the bench sheet as .5(10 x 12).

Example - Rhodomonas

CIRCLE



d = diameter r = radius

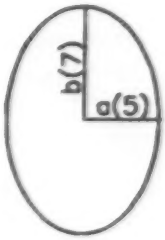
area = πr^2 *

This should be designated as (10^d) and you look up the value for the area of 10^d on the Table of Areas (p. 16-8).

Example - Coelastrum

* π = 3.14159265359

ELLIPSE



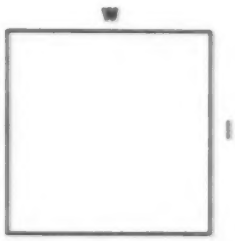
a = short radius b = long radius

Area = πab

This should be designated as (3.14159 x 5 x 7)ell. in the order in which they appear in the formula.

Example - Oocystis.

RECTANGLE OR SQUARE



l = length w = width

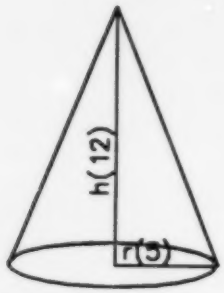
area = l x w

This should be designated as (60 x 3).

Example - Diatoma

6. Procedure and Formulae for Measuring the Volume of Geometric Shapes

CONE



h = height r = radius

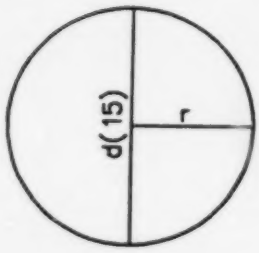
Volume = $\frac{1}{3}h(\pi r^2)$ or $1.047hr^2$

This should be designated as (1.047 x 12 x 5 x 5).

$\frac{\pi}{3}$ = 1.0471976

Example - Rhodomonas

SPHERE



d = diameter r = radius

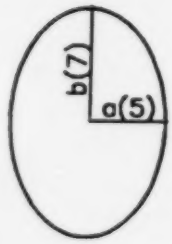
Volume = $\frac{4}{3}\pi r^3$

$\frac{4}{3}\pi$ = 4.1887902

This should be designated as (15^5). To find the value for the volume of a sphere, look at the top value opposite the appropriate diameter on the Table of Volumes (p. 16-9).

Example - Chlamydomonas (some)

ELLIPSE



a = short axis b = long axis

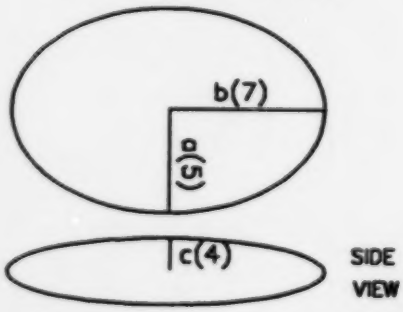
Volume = $4.18879a^2b$ or $\frac{4}{3}\pi a^2 b$

This should be designated as $(4.18879 \times 5^2 \times 7)$ell

$\frac{4}{3}\pi$ = 4.1887902

Example - Oocystis

FLATTENED ELLIPSE



a = short radius b = long radius

c = $\frac{1}{2}$ thickness

Volume = 4.18879abc

$\frac{4}{3}\pi$ = 4.1887902

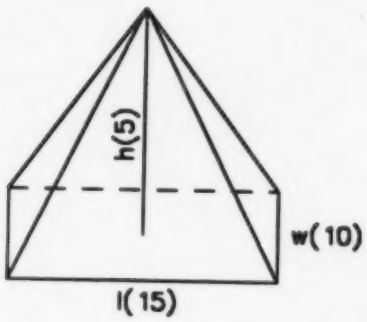
This should be designated as (4.18879 x 5 x 7 x 4) in the order in which it appears in the formula.

Example - Cryptomonas

CYLINDER

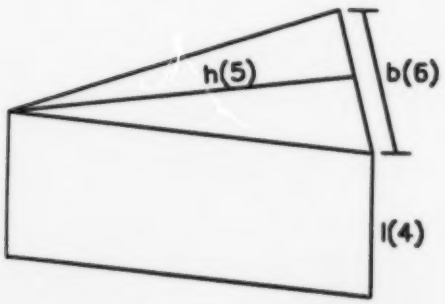
r = radius h = height
Volume = $(\pi r^2)h$
This should be designated as (15×4^d). The value of 4^d can be derived from the Table of Areas and Volumes.
Example - Mougeotia

PYRAMID



h = height l = length w = width
Volume = $\frac{1}{3}$(area of base x height)
This should be designated as .3333(15 x 10 x 8). Follow the order shown in the formula or $\frac{(l \times w \times h)}{3}$.

PRISM



b = base h = height l = length
Volume = area of base x length
$= \frac{1}{2}b \times h \times l$
This should be designated as .5(6 x 5 x 4).
Example - Navicula = double prism

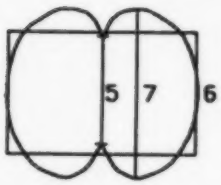
7. Phytoplankton Measurement - Problem Algae

CYANOPHYCEAE

Aphanothece

These cells are usually so small and packed in the colony that it is hard to measure them accurately. Individual size should be noted and then the colony should be clicked off as accurately as possible.

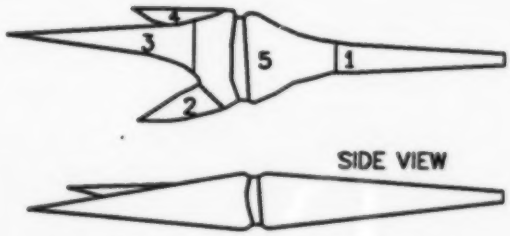
Anabaena



Cells often have partial indentations so they can't be counted as 2 spheres but instead as a cylinder. In this case it is better to take the average width between the width at the widest point and at the narrowest point. Mucilage around cells such as Chroococcus is not counted.

DINOPHYCEAE

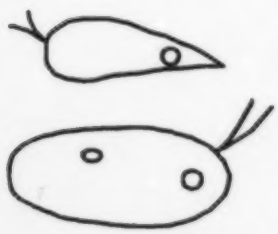
Ceratium



[(1)+(2)+(3)+(4)+(5)]

Ceratium is dorso-ventrally flattened. All measurements for 1 cell of Ceratium should be enclosed in square brackets. You should also be careful when you measure Peridinium types as they are very often flattened dorso-ventrally as well.

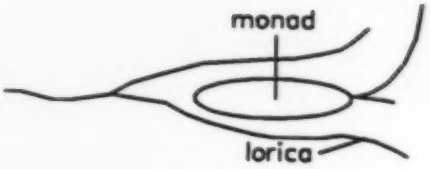
CRYPTOPHYCEAE



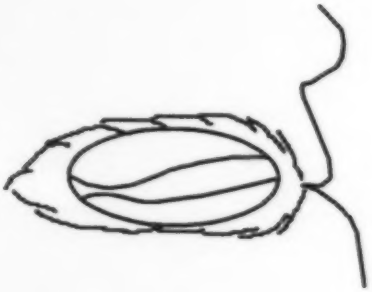
Rhodomonas is usually counted as a cone. Cryptomonas is a flattened ellipse. There is a special formula for this shape (see p. 15-8).

CHRYSOPHYCEAE

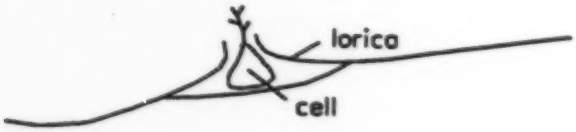
Dinobryon cysts. The whole cyst should be measured not just the contents.



Dinobryon - measure only the monad usually as a cylinder not the lorica.



Mallomonas - measure the whole cell out to the scaly cell wall as there is shrinkage of chloroplast resulting from preservation.

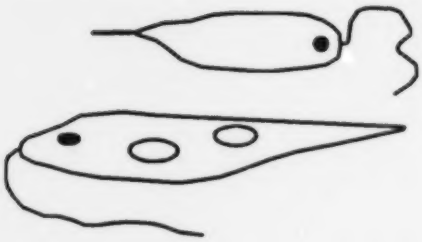


Bitrichia - measure only the cell and not the lorica.

Kephyrion - measure only the monad and not the lorica.

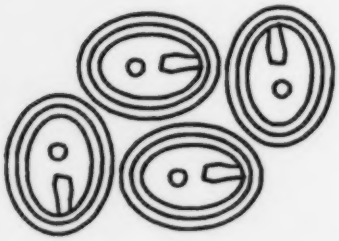
EUGLENOPHYCEAE

Phacus - flattened dorso-ventrally. Measure as a flattened disc or ellipse with a cone on the bottom.

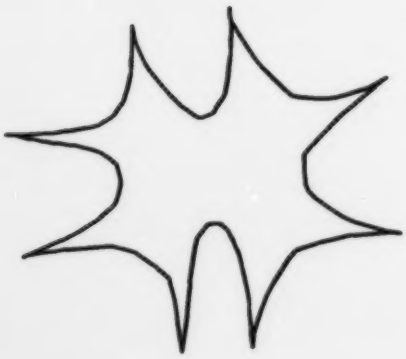


Euglena - has flexible pellicle so it is difficult to determine its thickness. It is often measured as two cones (double cone).

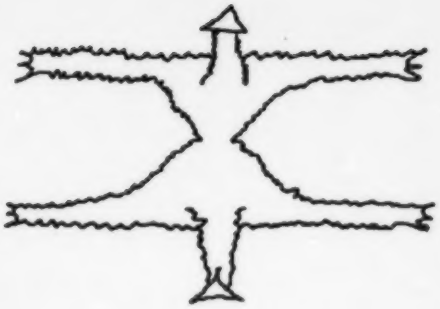
CHLOROPHYCEAE



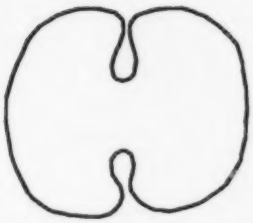
Gloeocystis - mucilage is not counted.



Arthrodesmus - These are usually counted as 2 flattened rectangles and the spines are counted as well.

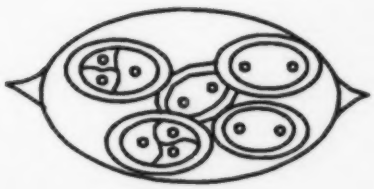


Staurastrum - Count the arms separately as cylinders and then count the body as a cylinder as well.

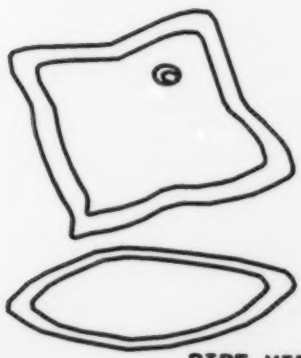


Cosmarium - can be counted as 2 cylinders or 2 ellipses depending on the shape.

Planctonema - measure only the cells and not the sheath.

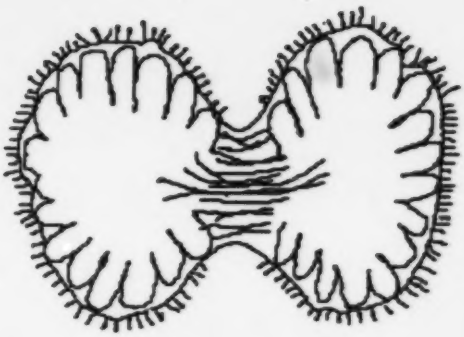


Oocystis - should be measured as an ellipse. Daughter cells inside an intact mother cell are counted as individuals.



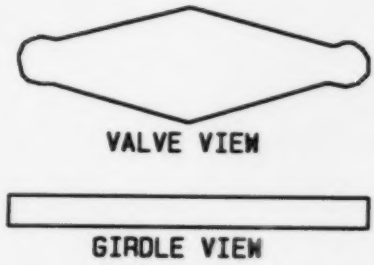
SIDE VIEW

Tetraëdron minimum - measure as a flattened square not as thick as it is wide.

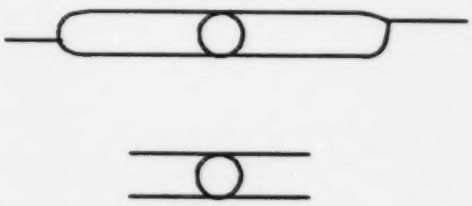


Botryococcus braunii - measure the whole colony including the mucilage. The cells are usually densely packed in the mucilage and it would be extremely difficult to measure individual cells.

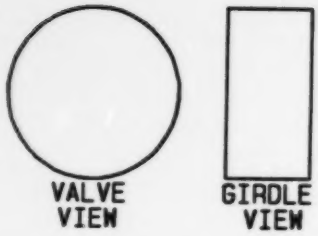
BACILLARIOPHYCEAE



Navicula - measure as 2 short prisms.

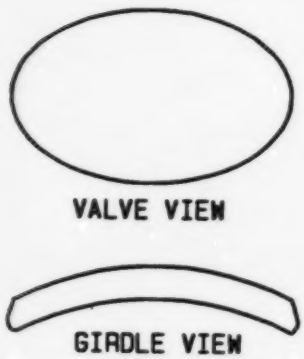


Rhizosolenia - if the cell is intact count it as a cylinder but if the ends are broken off count the chloroplast part only, in a section and note that the cells weren't intact.



Cyclotella - are usually seen in valve view and you can't see the thickness. If the cells are <6m in diameter assume the thickness to be half of the diameter. If cells are larger, you should try to flip one over after you have finished counting to see how thick they are.

Cymatopleura - can measure the ends as prisms and average the rest into a flattened rectangle. You must try and determine the thickness by flipping the cells over, especially with large diatoms.



Cocconeis - elliptical but flat. Use the formula for area of an ellipse x thickness of Cocconeis to get the volume.

In the event that an odd-shaped diatom is dominant in the sample it would be easier to take a sub-aliquot and put it on a flat slide to try and determine the third dimension.

8. Taxonomy

Identification to genus is generally required unless otherwise discussed. Some exceptions and other taxonomic notes are:

1) Unidentifiable forms. Fixation in Lugol's iodine distorts or destroys features used for identification of many forms. It is more useful to leave such questionable identifications as e.g. "unidentified species A" or other less descriptive term. However, provision should be made on the Bench Sheet to give some clues as to a higher taxonomic category if possible (e.g. how many flagella and relative lengths, filamentous, colonial or single-celled, autospores present, etc.,). Remember that there are many armoured dinoflagellate genera and identifying something as "unident. armoured dinoflagellate" is better than assigning a genus name with uncertainty.

2) If the analyst is certain of a species identification, the species name should be recorded on the bench sheet and card. Many species of Anabaena, Dinobryon, Chrysolykos, Melosira, etc., cannot be confused with any others in the fixed state and you are encouraged to use species names when they are known.

3) A distinction should be made between Chrysochromulina parva and C. breviturrita in all samples. This is very important because C. breviturrita is a serious odour producer, while C. parva is not. Reference samples containing large amounts of C. parva and C. breviturrita are available to all staff to confirm the identity of these taxa.

4) A distinction is being made between Chrysosphaerella longispina and C. coronacircumspina. C. coronacircumspina is difficult to distinguish from one or two species of Spiniferomonas with the light microscope. You may wish to group these organisms under one heading, i.e. "Spiniferomonas/Chrysos. coronacircumspina".

5) We have been "using" Stephanodiscus bineranus instead of Melosira binderana for some time now. This is to continue; make sure the full name is recorded (genus + species).

6) Diceras is an invalid name. The species previously grouped under Diceras are properly assigned to Bitrichia. These are quite different from Chrysolykos spp. (Chrysoikos = Syn. Chrysolykos).

7) For our purposes, "Kephyrion" will continue to include species of Kephyrion as well as Pseudokephyrion. It is better to use the heading "Kephyrion/Pseudok".

LABORATORY - PLANKTON ENUMERATION - QUANTITATIVE

Using your own microscope, if you have one fitted with a suitable ocular micrometer, undertake a plankton count in one strip across the S-R cell, using the areal standard unit method. Record your count on a bench sheet.

Check with one of the instructors present to ensure that you are using the proper multiplier factor for your microscope.

Count a single strip using the sample which is available and which has previously been counted in our laboratory. Use a bench sheet and then record your count on a report form.

Continue practising counts using your own water sample or any of the other samples which are available. Compare results of your counts with our records.

COUNTING PROCEDURES

Shake the sample thoroughly to evenly distribute the plankton in the sample, but not so hard that individual colonies will be disrupted.

Concentrate the sample in accordance with the instructions provided in Section 11.

Transfer 1 mL of the sample to the S-R cell as described previously. Remove the aliquot from the sample bottle before the organisms have time to settle to the bottom. After the cover glass rotates properly into position, wipe off excess water lightly with a tissue. The cover glass should remain intact on the S-R even when turned upside down.

Set the S-R cell on the microscope stage and allow to stand for several minutes to enable the organisms to settle to the bottom of the counting cell.

Select ten randomly distributed fields or one strip for the count.

In a concentrated count, include all of the organisms lying within the Whipple field (see P. 12-4).

All the organisms lying between the ends of the vertical lines in the ocular grid should be included in a one strip count. Organisms which lie across the ends of these lines should be counted at the top, but not at the bottom if counting by strips.

Note: Examples of a "Bench Sheet" and a "Plankton Enumeration Sheet" are provided on page 20-2 and page 20-3.

For each field counted, identify and measure the individual cells and colonies as they are encountered by means of the lines and

squares making up the ocular grid. The fine adjustment knob should be turned so that you can determine whether all of the organisms in each field are resting on the bottom. Any other organism observed at various levels must be included in the count. Determine the areal value for each cell or colony as they are observed and record on the bench sheet.

When your tally is completed for the ten fields or entire strip, multiply the total for each genus by the required multiplier factor (F) to give you the total areal value for each in the entire millilitre in the counting cell. Divide the total for each genus by 400 to obtain the number of areal standard units of each genus per millilitre. Always remember, when the sample has been concentrated, the total must be further divided by the appropriate concentration factor (f).

A "survey count" should be made using low power over half of the counting cell to determine the numbers of animal plankton present. These are recorded by number rather than by assigning areal values.

Calculation Formulae:

The appropriate calculation to convert to numbers per millilitre is as follows:

$$\frac{\text{number of organisms X Microscope Factor (F)}}{\text{concentration factor(f)}} = \text{No. / mL}$$

The appropriate calculation to convert to A.S.U. per millilitre is as follows:

$$\frac{\text{Total Square Microns X Microscope Factor (F)}}{400 \quad \text{X concent. factor (f)}} = \text{A.S.U./mL}$$

The appropriate calculation to convert to volumetric measurement is as follows:

$$\frac{\text{Total Cubic Microns X Microscope Factor (F)}}{1000 \quad \text{X concent. factor (f)}} = \text{cu. microns/mL}$$

*NOTE: The micron is now an obsolete term. The correct International System (SI) terminology is the micrometer so that 1 micron = 1 micrometer and 1 cubic micron = 1 cubic micrometer, which, written in symbols, is $1\mu = 1\mu m$; $1\mu^3 = 1\mu m^3$.

In reporting μm units on our Bench Sheets we divide by 1000 to avoid recording large numbers ie. $165{,}259\mu m^3$ /mL is recorded as 165.26×10^3 on Bench Sheet and transferred to Analysis Report card as 165 x 1000 μm^3 /mL. (see P. 20-5, 20-6)

Units are recorded on the Bench Sheet as $\mu m^3 \times 10^{-3}/mL$. (see P.20-5 last column).

These units are recorded on the Analysis Cards as μm^3 x 1000 /mL. (see P.20-6).

These units are often recorded in our reports and journal papers as mm^3/L.

This is derived from:

$1\ mm^3/L = 1.0 \times 10^9\ \mu m^3/L$

$1.0 \times 10^9\ \mu m^3/L = 1.0 \times 10^9\ \mu m^3/1000\ mL$

$1.0 \times 10^9\ \mu m^3/1000\ mL = 1.0 \times 10^6\ \mu m^3/mL$

$\therefore 1\ mm^3/L = 1.0 \times 10^6\ \mu m^3/mL$

Interpretation of Algal Densities*

	Low	Moderate	High
ASU/mL	<200	200-2000	>2000
mm^3/L	0.5-1.0	1.5-3.5	>3.5

*Report: The Kawartha Lakes Water Management Study - Water Quality Assessment (1972-1976). November 1976. Ministry of the Environment/Ministry of Natural Resources. pp.185.

LABORATORY - TEST SAMPLES RELATED TO ENUMERATION

Your microscope has been centred on one particular cell, colony or filament of algae. Assign what you believe to be the correct areal value to your organism and record the areal value and the number of the microscope. Return the organism to its original position when you finish. Go to all of the other microscopes in turn and repeat, recording the areal values and numbers of the microscopes as you proceed.

From the sample which has been provided, do an areal count involving ten fields and calculate the appropriate multiplier factor which you must use to project to areal standard units per millilitre.

BENCH SHEET - PLANKTON ENUMERATION

Sample No. _____

Source (Lake): _____ Date Analyzed: _____ Date Sampled: _____ P___ of ___

Location (Station): _____ Counted By: _____ Micr. Factor: _____ =

Depth: _____ Count Procedure: _____ Conc. (f) x (combined factor)

Taxonomic Name		Total ____ microns	Total ___per mL A. S. U. /cu. mic
	Check Microns =	Total _____ = per mL	

SUMMARY SHEET - PLANKTON ENUMERATION

Municipality: _____ Source: _____ Sample No. _____

Sampling Point (Raw Water etc.): _____ Date Sampled _____

Analyzed by: _____ Date Analyzed _____

Enumeration Procedure Use: _____ Concentration Factor _____

Enumeration Factor - Algae: _____ Enumeration Factor - Zooplankton _____

ALGAE Taxonomic Group	Total Sq. Microns	A.S.U. Per ml.	
Blue Greens			
	B.G. Total		
Greens			
	Green Total		
Flagellates			
	Flag. Total		
Diatoms			
	Diatom Total		
TOTAL A.S.U. PER ML.			

ZOOPLANKTON Taxonomic Group	Total Pieces	No. per ml.
PROTOZOA		
Ciliates		
Flagellates		
Sarcodina		
Total No. Per ml.		
MICRO-INVERTEBRATES		
Rotifers		
Total No. Per ml.		
CRUSTACEA		
Cyclops		
Daphnia		
Total No. Per ml.		
MISCELLANEOUS		
Total No. Per ml.		
Total No. ZOOPLANKTON Per ml.		

DIAGRAM - UNKNOWNS

WATERWORKS INFORMATION

Raw H_2O Temp. _____

Raw H_2O Turbidity _____

Raw H_2O Odour (if present) _____

Threshold Odour No. _____

Microstrainers: in/out of service

Weather: (if unusual and affecting intake) _____

Air Temp. _____

Filter runs: _____ hrs.

Remarks: _____

PLANKTON ANALYSIS

FILE NUMBER

SAMPLE NUMBER

Municipality _____ Date Analysed _____ Date Sampled _____

Source _____ Enumeration Procedure _____

Station _____ Depth _____ Mic Factor _____ Concentration Factor _____

CYANOPHYCEAE			DINOPHYCEAE			CHRYSOPHYCEAE		
			TOTAL →					
			CRYPTOPHYCEAE					
			TOTAL →					
			EUGLENOPHYCEAE					
TOTAL →			TOTAL →			TOTAL →		

CHLOROPHYCEAE			BACILLARIOPHYCEAE			ZOOPLANKTON	No. Organisms per ML
						TOTAL →	
TOTAL →			TOTAL →				

REMARKS:

SIGNED: _____

OR TOTAL AREAL STANDARD UNITS PER ML →

TOTAL CUBIC MICRONS PER ML

MOE 0000 6/82

AREA OF CIRCLES IN SQUARE MICROMETERS IF GIVEN THE DIAMETER

DIAMETER 1-10	AREA	DIAMETER 11-20	AREA	DIAMETER 21-30	AREA	DIAMETER 31-40	AREA	DIAMETER 41-50	AREA
1	.785	11	95	21	346	31	755	41	1320
2	3.14	12	113	22	380	32	804	42	1385
3	7.07	13	133	23	416	33	855	43	1452
4	12.57	14	154	24	452	34	908	44	1521
5	19.63	15	177	25	491	35	962	45	1590
6	28.27	16	201	26	531	36	1018	46	1662
7	38.48	17	227	27	573	37	1075	47	1735
8	50.27	18	255	28	616	38	1134	48	1810
9	63.62	19	284	29	661	39	1195	49	1886
10	78.54	20	314	30	707	40	1257	50	1964

DIAMETER 51-60	AREA	DIAMETER 61-70	AREA	DIAMETER 71-80	AREA	DIAMETER 81-90	AREA	DIAMETER 91-100	AREA
51	2043	61	2923	71	3959	81	5153	91	6504
52	2124	62	3019	72	4072	82	5281	92	6648
53	2206	63	3117	73	4185	83	5411	93	6793
54	2290	64	3217	74	4301	84	5542	94	6940
55	2376	65	3318	75	4418	85	5675	95	7088
56	2463	66	3421	76	4537	86	5809	96	7238
57	2552	67	3526	77	4657	87	5945	97	7390
58	2642	68	3632	78	4778	88	6082	98	7543
59	2734	69	3739	79	4902	89	6221	99	7698
60	2827	70	3849	80	5027	90	6362	100	7854

VOLUME OF SPHERES IN CUBIC MICROMETERS IF GIVEN THE DIAMETER

DIAMETER 1-10	VOLUME	DIAMETER 11-20	VOLUME	DIAMETER 21-30	VOLUME	DIAMETER 31-40	VOLUME	DIAMETER 41-50	VOLUME
1	0.52	11	697	21	4848	31	15596	41	36080
2	4.19	12	905	22	5574	32	17154	42	38785
3	14.13	13	1150	23	6369	33	18813	43	41622
4	33.50	14	1435	24	7237	34	20576	44	44594
5	65.44	15	1767	25	8180	35	22445	45	47704
6	113.08	16	2144	26	9201	36	24424	46	50955
7	179.56	17	2572	27	10304	37	26517	47	54351
8	268.03	18	3053	28	11492	38	28725	48	57895
9	381.63	19	3591	29	12768	39	31054	49	61589
10	523.50	20	4188	30	14135	40	33504	50	65438

DIAMETER 51-60	VOLUME	DIAMETER 61-70	VOLUME	DIAMETER 71-80	VOLUME	DIAMETER 81-90	VOLUME	DIAMETER 91-100	VOLUME
51	69443	61	118825	71	187366	81	278209	91	394494
52	73608	62	124765	72	195395	82	288641	92	407643
53	77937	63	130900	73	203650	83	299330	93	421081
54	82432	64	137232	74	212135	84	310281	94	434811
55	87097	65	143766	75	220852	85	321494	95	448836
56	91935	66	150504	76	229804	86	332975	96	463159
57	96949	67	157449	77	238995	87	344726	97	477784
58	102131	68	164605	78	248428	88	256751	98	492714
59	107516	69	171974	79	258106	89	369051	99	507952
60	113076	70	179561	80	268032	90	381632	100	523500

TYPICAL FACTORS FOR OLYMPUS MICROSCOPES

Area Examined (read down)	Microscope Factor*(F)	Divide by 400	Concentraction Factors (f) (read across top)			
			1	10	20	40
			Microscope Factor Divided by (400 X Concentration Factor)			
1/4 Strip	129.04	400 =	0.322	0.032	0.016	0.008
1/2 Strip	64.52		0.16	0.016	0.008	0.004
3/4 Strip	43.01		0.12	0.012	0.006	0.003
1 Strip	32.26		0.08	0.008	0.004	0.002
2 Strips	16.13		0.04	0.004	0.002	0.001
3 Strips	10.75		0.027	0.0027	0.00135	0.0007
4 Strips	8.065		0.02	0.0020	0.0010	0.0005
5 Strips	6.452		0.016	0.0016	0.0008	0.0004
1/2 Cell	2.0		0.005	0.0005	0.00025	0.00013
1 Field	8163.3		20.4	2.04	1.02	0.51
5 Fields	1632.66		4.08	0.408	0.204	0.102
10 Fields	816.33		2.04	0.204	0.102	0.051
15 Fields	544.22		1.36	0.136	0.068	0.034
20 Fields	408.165		1.02	0.102	0.051	0.0255
40 Fields	204.08		0.51	0.051	0.0255	0.0128

* To convert area examined to area per mL.

Formula to convert total square microns to Areal Standard Units per mL (ASU/mL).

$$\frac{\text{Total Square Microns X (F)}}{\text{400 X Conc. (f)}}$$

TYPICAL FACTORS FOR NIKON INVERTED MICROSCOPES

Area Examined (read down)	Microscope Factor (F)	Divide by 1000	Concentration Factors (f) (read across top)			
			1	4	10	20
			Microscope Factor Divided by (1000 X Concentration Factor)			
1 radius	170.65	1000 =	0.171	0.043	0.0171	0.0085
2 radii	85.33		0.085	0.021	0.0085	0.0043
3 radii	56.88		0.057	0.014	0.0057	0.0028
4 radii	42.66		0.043	0.011	0.0043	0.0021
5 radii	34.13		0.034	0.0085	0.0034	0.0017
6 radii	28.44		0.028	0.0071	0.0028	0.0014
7 radii	24.38		0.024	0.0061	0.0024	0.0012
8 radii	21.33		0.021	0.0053	0.0021	0.0011
9 radii	18.96		0.019	0.0047	0.0019	0.00095
10 radii	17.06		0.017	0.0043	0.0017	0.00085
12 radii	14.22		0.042	0.0036	0.0014	0.00071
15 radii	11.37		0.011	0.0028	0.0011	0.00057
20 radii	8.53		0.0085	0.0021	0.00085	0.00043
1/2 chamber	2.00		0.002	0.0005	0.0002	0.0001
1 chamber	1.00		0.001	0.00025	0.0001	0.00005

Formula to convert total cubic microns to Biovolume: Cubic micometers per mL ($\mu m^3 x 10^{-3}/mL$)

$$\frac{\text{Total Cubic Microns X F (Microscope Factor)}}{\text{1000 X f (concentration factor)}}$$

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APPENDIX A

INTRODUCTION TO ALGAL TAXONOMY AND CLASSIFICATION[1]

The question of the phylogenetic relationships between the algae rests upon the discovery of an adequate basis for classifying them. The test of time has shown the inadequacy of taxonomic classifications of algae based either upon the organization of the plant body or upon the method of reproduction. It has become increasingly clear during the past quarter century that the morphology and physiology of the individual cells are the fundamental basis upon which the algae must be classified. The evidence has shown that there are several classes among the algae, each of which has cells with certain distinctive morphological and physiological traits. Chief among the morphological characteristics is the structure of the motile cell, and for most of the algal classes, there is a striking constancy in its organization, especially with respect to the number, arrangement, relative length and cytological structure of the flagella. On the physiological side, there is, throughout each class, a constancy in the pigments present in the plastids and a constancy in the chemical nature of food reserves accumulating through photosynthetic activity. For example, the Chlorophyceae always have flagella of equal length, a predominance of green pigments in their plastids, and usually store photosynthetic reserves as starch. The Xanthophyceae on the other hand, always have flagella of unequal length, a predominance of yellow pigments in their plastids, no formation of starch, and usually store photosynthetic reserves as oils.

Experience in water and sewage treatment plants has demonstrated that considerable difficulty exists in recognizing the various algae that are encountered and in determining which of the many present are really important. In this "Phytoplankton Methods Manual", the forms are considered and displayed according to their significance to environmental scientists and technicians, not as botanists would normally classify them. However, it is essential to realize that the four main groups (Diatoms, Greens, Blue-greens and Flagellates) described in the manual may be phylogenetically and taxonomically divided into thirteen classes. These are the Bacillariophyceae, Chlorophyceae, Chrysophyceae, Cryptophyceae, Dinophyceae, Euglenophyceae, Cyanophyceae, Prasinophyceae, Raphidiophyceae, Xanthophyceae, Prymnesiophyceae, Phaeophyceae and Rhodophyceae.

Note 1: The general description for each of the eight classes throughout this appendix is extracted from "Freshwater Algae of the United States" (Smith, 1950) and "Les Algues d'eau douce" (Bourelly, 1972, 1981, 1985) while the class and order structure follow Bourrelly with updates to 1990.

Studies carried out over the last few years by members of the Aquatic Plant Unit, M.O.E. have indicated that all of the phytoplanktonic forms and most of the attached algae in Southern Ontario waters which are significant to environmental scientists and waterworks operators can be included in eight of these thirteen classes.

A brief description of the classes, their main orders and genera are as follows:

CHLOROPHYCEAE

The Chlorophyceae, or "grass-green" algae, have their photosynthetic pigments localized in chloroplasts which are "grass-green" because of the predominance of chlorophylls a and b over the carotenes and xanthophylls. There are several xanthophylls not found in other algae, and of these, lutein is the most abundant. Photosynthetic reserves are usually stored as starch, and its formation is intimately associated with an organ of the chloroplast, the pyrenoid. Motile stages have flagella of equal length and with a few exceptions, the zoospores and motile gametes have two or four flagella. Although sexual reproduction is not a feature distinguishing Chlorophyceae from other algae, it is a phenomenon of wide occurrence within the group.

Almost every sample of fresh surface water contains at least a few representatives of this division. In quiet waters, the filamentous species are most conspicuous, but there are often many unicellular and colonial species intermingled with them. In most cases, the pools contain small sessile species, either upon the coarser algae, upon submerged stems and leaves, or upon stones or woodwork in the water. Temporary pools and puddles are usually not so rich in green algae as are pools of a more permanent nature, and one is more apt to find Cyanophyceae than Chlorophyceae in such habitats.

Although the total biovolume of Chlorophyceae is rarely predominating in the phytoplankton of ponds and lakes, the number of species of chlorophytes in the fresh-water plankton is often very large. The number of genera known only from the plankton is greater in this division than in any other. As a general rule, planktonic Chlorophyceae are most abundant during late spring and early autumn, but this periodicity is not sharply marked, and they are to be found at all times when plankton can be sampled from ponds and lakes.

The following forms commonly occur in Ontario waters:

Class: Chlorophyceae

1. Order: Volvocales

Genera:
- Carteria
- Chlamydomonas
- Eudorina
- Gonium
- Pandorina
- Pleodorina
- Volvox

2. Order: Tetrasporales

Genus: Tetraspora

3. Order: Chlorococcales

Genera:
- Actinastrum
- Ankistrodesmus
- Asterococcus
- Botryococcus
- Chlorella
- Chlorococcum
- Closteriopsis
- Coelastrum
- Crucigenia
- Dactylococcus
- Dictyosphaerium
- Dimorphococcus
- Echinosphaerella
- Franceia
- Gloeocystis
- Golenkinia
- Hydrodictyon
- Kirchneriella
- Lagerheimia
- Micractinium
- Monoraphidium
- Nephrocytium
- Oocystis
- Pediastrum
- Planktosphaeria
- Quadrigula
- Scenedesmus
- Selenastrum
- Sphaerocystis
- Tetraedron
- Tetrastrum

4.	Order:	Ulotrichales
	Genus:	Ulothrix
5.	Order:	Ulvales
6.	Order:	Chaetophorales
	Genera:	Chaetophora Draparnaldia Stigeoclonium
7.	Order:	Trentepohliales
8.	Order:	Oedogoniales
	Genera:	Bulbochaete Oedogonium
9.	Order:	Sphaeropleales
10.	Order:	Siphonocladales
	Genera:	Cladophora Pithophora Rhizoclonium
11.	Order:	Siphonales
12.	Order:	Dichotomosiphonales
	Genus:	Dichotomosiphon
13.	Order:	Zygnematales
	Family:	Zygnemataceae
	Genera:	Mougeotia Spirogyra Zygnema Zygogonium
	Family:	Mesotaeniaceae
	Genera:	Mesotaenium Netrium Roya

Family: Desmidiaceae

Genera: Arthrodesmus
Closterium
Cosmarium
Desmidium
Euastrum
Micrasterias
Staurastrum

CHRYSOPHYCEAE

Cells of this class have chloroplasts of a distinctive golden-brown colour because of a predominance of beta carotene and certain xanthophylls. The chief photosynthetic reserves are leucosin, an insoluble compound of unknown composition but thought to be a carbohydrate, and oils. According to the genus, motile vegetative and reproductive cells have one flagellum, or two flagella of equal or unequal length.

Many fresh-water Chrysophyceae are restricted to cold brooks, especially mountain streams, and to springs. Others are found in the plankton of lakes and in greatest abundance during spring and autumn when the water is cool, and most thrive best in water relatively free from impurities.

Class: Chrysophyceae

1. Order: Phaeoplacales

2. Order: Stichogloeales

3. Order: Chrysosaccales

4. Order: Rhizochrysidales

   Genera: Bitrichia
   Chrysidiastrum

5. Order: Chromulinales

   Genera: Bicosoeca[1]
   Chromulina
   Chrysamoeba
   Chrysocapsa
   Kephyrion

[1] Some taxonomists place this genus in a separate order Bicosoecales

6. Order: Ochromonadales

Genera:
Chrysolykos
Chrysosphaerella
Dinobryon
Mallomonas
Ochromonas
Spiniferomonas
Synura
Uroglena

Order: Monosigales[1] (Craspedomonadaceae)

Genera:
Codonocladium
Salpingoeca
Stelexomonas

[1]: These genera are more correctly placed in the zoological class Choanoflagellida. Bourrelly places them as an order Monosigales in the chrysophytes. We include them here with the chrysophytes for convenience.

PRYMNESIOPHYCEAE

There is only one freshwater genus present in the order Prymnesiales in Ontario represented by Chrysochromulina spp. While the class has many of the characteristic features of the golden-brown flagellates the genus is unique in that it has two flagella plus one haptonema (a rigid filiform organ lying between the flagella). It is important to the taxonomists identifying these genera in water supplies as C. breviturrita is a notorious odour producer whereas C. parva is not.

Class: Prymnesiophyceae

Order: Prymnesiales

Genus: Chrysochromulina

BACILLARIOPHYCEAE

The Bacillariophyceae, or diatoms, include a large number of unicellular and colonial genera that differ sharply from other algae in the shape of their cells. The primary feature distinguishing diatoms from other algae is that the cell wall is highly silicified and composed of two overlapping halves that fit together as to the two parts of a Petri dish. The siliceous nature of the cell wall cannot be determined by microscopical examination, but diatoms may be readily recognized by the bilateral or radial markings on the wall when a cell is viewed from above. Within a cell are one to many, variously shaped, yellowish to brownish chloroplasts, which contain chlorophyll a, chlorophyll c, betacarotene, a unique carotene, and xanthophylls, certain of which are found only in the Bacillariophyceae.

Diatoms are especially common during the spring and autumn. Fresh-water diatoms are found in a wide variety of habitats, although the greater number of them are strictly aquatic. A few of these aquatic species are found only in the plankton of ponds and lakes, where during the spring and autumn, they may be present in sufficient quantity to give the water a distinctly fishy odour. Diatoms can also be the most abundant form in mid-summer in shallow eutrophic waters (eg. Bay of Quinte). The algal flora of roadside ditches, semipermanent or permanent pools, and streams of rivulets always includes some diatoms. When growing in standing water, diatoms are often to be found either as a brownish sludge on the bottom of a pool, or as a coating on stems and leaves of water plants. Also, they grow intermingled with or epiphytic upon other algae, and the filaments of Cladophora, Rhizoclonium and Vaucheria are often thickly covered with certain of the sessile species. When diatoms grow in rapidly flowing water, they occur mostly in a gelatinous matrix, coating rocks and stones in the bed of the stream.

Class: Bacillariophyceae

1. Order: Centrales

Genera:
- Attheya
- Coscinodiscus
- Cyclotella
- Melosira
- Rhizosolenia
- Stephanodiscus

2. Order: Pennales

Genera:
- Amphiprora
- Amphora
- Asterionella
- Caloneis
- Cocconeis

Cymatopleura
Cymbella
Denticula
Diatoma
Epithemia
Eunotia
Fragilaria
Gyrosigma
Meridion
Navicula
Nitzchia
Opephora
Pinnularia
Pleurosigma
Stauroneis
Surirella
Synedra
Tabellaria

EUGLENOPHYCEAE

Most members of the division are free-swimming cells with one, two or three flagella. Many of the genera have grass green, discoid, band-shaped or stellate chloroplasts, with or without pyrenoids. The chloroplasts contain the same chlorophylls as Chlorophyceae, beta-carotene only, and at least one xanthophyll not found in Chlorophyceae. The nutrition may be holophytic, holozoic, or saprophytic, but irrespective of the mode of nutrition, the food reserved are paramylum (an insoluble carbohydrate related to starch) and fats. There are one or more contractile vacuoles at the anterior end of a motile cell, which are connected with a reservoir, which in turn, is connected with the cell's exterior by a narrow gullet. Thick-walled resting stages (cysts) are known for several genera.

Euglenoids are most often found in small pools rich in organic matter. The pigmented forms, especially Euglena are frequently present in sufficient abundance to colour the water. The saprophytic colourless forms are rarely present in quantity and they grow most abundantly when a considerable amount of putrefaction is taking place. Sessile species grow upon algae, plant debris, and small planktonic crustaceans.

Class: Euglenophyceae

1. Order: Euglenales

Genera: Euglena
Lepocinclis
Phacus
Trachelomonas

DINOPHYCEAE

The most distinctive feature of the class is the structure of motile vegetative cells and of zoospores of immobile genera. These are always completely or incompletely encircled by a transverse or by a spiral groove. Motile cells are always biflagellate and the two flagella inserted in the groove. One flagellum lies in the groove and encircles the cell; the other extends backward from the groove. Most members of the class have brownish chloroplasts, but some are colourless and with a saprophytic or holozoic mode of nutrition. A few genera have naked protoplasts, but the great majority have cellulose walls that may be homogenous or may consist of a definite number of articulated plates. Food reserves are stored as starch or as oil. Reproduction of motile genera is usually by vegetative cell division, either while a cell is in motion or after it has come to rest. Motile genera may also produce aplanospores (cysts). Reproduction of immobile genera may be by means of zoospores or autospores (aplanospores). Sexual reproduction is infrequent.

The great majority of the Dinophyceae are motile unicellular flagellates (dinoflagellates). Freshwater dinoflagellates are most abundant in pools, ditches, and small lakes with considerable vegetation. They are not uncommon in plankton samples from large lakes, but rarely occur in abundance. Some of the fresh-water species thrive best in hard waters; others are found in greatest numbers in soft waters.

Class: Dinophyceae

1. Order: Desmomastigales

2. Order: Prorocentrales

3. Order: Peridiniales

Genera: Ceratium
Diplosalis
Glenodinium
Gymnodinium
Peridinium

4. Order: Dinococcales

CRYPTOPHYCEAE

Protoplasts of the Cryptophyceae usually contain two chloroplasts that are brownish in most cases but may be red, blue, blue-green or grass-green. Reserve foods are usually stored as starch or starch-like compounds. Most of the Cryptophyceae are unicellular flagellates with asymmetrically compressed cells, surrounded by a firm periplast, and with two flagella slightly different in length. Insertion of the flagella may be terminal or lateral.

Some fresh-water cryptomonads grow in waters rich in organic and in nitrogenous materials; others grow in waters with small amounts of these materials.

Class: Cryptophyceae

1. Order: Cryptomonadales

Genus: Cryptaulax
Cryptomonas
Rhodomonas
Katablepharis

CYANOPHYCEAE

The Cyanophyceae, or blue-green algae, are a distinctive group sharply delimited from other algae in a number of respects. They are the only algae in which the pigments are not localized in definite chloroplasts. The pigments are localized in the peripheral portion of the protoplast and include chlorophyll a, carotenes, and distinctive xanthophylls. In addition, there is a blue pigment (c-phycocyanin) and a red pigment (c-phycoerythrin). Another unique feature of Cyanophyceae, is the primitive type of nucleus, the central body which lacks a nucleolus and a nuclear membrane. Equally important, although negative in character, are the lack of flagellated cells and the total lack of gametic union in all members of the division.

Cyanophyceae are found in a wide variety of habitats, and one can rarely examine a collection of fresh-water algae without encountering at least a few individuals of the class. Samples taken from permanent or semi-permanent pools usually contain representatives of both the filamentous and the non-filamentous genera, but neither type is ordinarily the dominant alga of the station. At times, one may one encounter a pool which is practically a pure culture of some blue-green alga, usually an Anabaena or an Oscillatoria species. On the other hand, temporary pools and puddles that have been standing but a week or two usually have an algal flora

composed entirely of blue-green algae, and one in which a single species frequently predominates. Species of Oscillatoria and Phormidium are most frequently encountered in such pools.

Blue-green algae are always present in plankton samples from fresh-water lakes and ponds. These are usually species of Chroococcus, and Coelosphaerium, or Anabaena. The proportion of Cyanophyceae with respect to other algae is dependent on both the time of the year and the chemical composition of the water. The blue-greens usually occur in abundance only during the warm months of the year, but certain species, such as Oscillatoria prolifica (Grev.) Gom., may also develop in quantity during the winter. Soft-water lakes ordinarily have more Chroococcales than other Cyanophyceae, but these never predominate at any season of the year. Hard-water lakes, on the other hand, exhibit a pronounced seasonal variation in the volume and percentage of Cyanophyceae, which are usually the dominant organisms during the late summer and early fall. Not infrequently, in such lakes, there is a development of one or two species to such an extent that the water is discoloured by them. This water bloom, which may also be caused by algae of other classes, may be of sporadic occurrence or may occur annually. In the case of some small ponds in California, the water is in "bloom" throughout the year. Instances have been recorded where the blooming of a lake has caused the death of fish or where the water is injurious to livestock drinking it, but such effects are very uncommon. The disagreeable tastes and odours caused by the death and decay of the algae causing the bloom are of far greater economic importance, especially in lakes and reservoirs used for domestic water supplies, and environmental engineers have studied at length the problem of eradicating algal growths.

Class: Cyanophyceae

1. Order: Chroococcales

Genera:
Aphanocapsa
Aphanothece
Chroococcus
Coelosphaerium
Dactylococcopsis
Gloeocapsa
Gloeothece
Gomphosphaeria
Merismopedia
Microcystis
Rhabdoderma
Synechoccocus

2. Order: Pleurocapsales

3. Order: Chamaesiphonales

4. Order: Stigonematales

5. Order: Nostocales (= Oscillatoriales)

Genera:

Anabaena
Aphanizomenon
Arthrospira
Gloeotrichia
Lyngbya
Nostoc
Oscillatoria
Rivularia
Scytonema
Spirulina
Tolypothrix

GLOSSARY OF TERMS

Aplanospores: non-motile asexual spores, formed one to several in but not the same shape as a parent cell.

Carotene: an orange-coloured pigment, usually associated with chlorophyll.

Cellulose: a carbohydrate forming main part of plant cell walls, $(C_6 H_{10} O_5)_n$

Chloroplast: a body (plastid) in the cell containing chlorophyll as the predominating pigment.

Chlorophyll: the green colouring matter in plants, partly responsible for photosynthesis.

Chromatophore: a coloured body in a cell which has a pigment other than chlorophyll predominating.

Contractile vacuole: a small spherical vesicle, found in cytoplasm of many single-celled forms, with excretory or hydrostatic function.

Crustacea: mostly aquatic animals with rigid outer coverings, joined appendages and gills. Examples are crayfish, Amphipoda (scuds), Copepoda (Cyclops) and Cladocera (water fleas).

Flagellum: a stout, whip-like organ of locomotion which arises within the cell.

Gamete: a sex cell, male or female reproductive cell.

Holophytic: obtaining food by photosynthesis.

Holozoic: ingesting food like an animal.

Leucosin: a white food reserve material found in most Heterokonate (blue-green with heterocysts)

Morphology: the science of form and structure of plants and animals, as distinct from consideration of functions.

Nucleus: complex spheroidal mass essential to life of most cells.

Nucleolus:	a dense rounded mass in a cell nucleus, consisting of protein and ribonucleic acid granules, and functioning in RNA and protein synthesis.
Paramylon:	a solid carbohydrate food reserve formed by certain euglenoids.
Periplast:	the bounding membrane, especially the cell membrane of euglenoids.
Photosynthesis:	the process by which simple sugars are manufactured from carbon dioxide and water by living plant cells with the aid of chlorophyll in the presence of sunlight.
Phylogenetic:	racial or historical development of plants of animals.
Phytoplankton:	plant micro-organisms, such as certain algae, living unattached in the water.
Physiology:	that part of biology dealing with functions and activities of organisms.
Plankton:	free-floating organisms unable to swim against currents, drifting.
Plastids:	any one of several kinds of bodies in the cytoplasm of a cell.
Protoplasts:	the living material (protoplasm) of a cell.
Putrefaction:	the decomposition of proteins by anaerobic micro-organisms.
Pyrenoid:	a protein granule which collects starch, either within a chloroplast, on its surface, or free within the cytoplasm.
Saprophyte:	organisms that obtain food from dead organic matter.
Stellate:	star shaped.
Taxonomy:	the system of classification as applied to natural history.
Vegetative cell division:	reproduction by bud-formation or other asexual method in plants and animals.

Xanthophyll: yellow pigment associated with chlorophyll.

Zoospore: a motile cell normally produced by a thallus or filament but not capable of sexual reproduction.

APPENDIX B - Section 20

Examples of Completed Forms
Used in Quantitative Enumeration

Microscope Calibration Chart for completed Counts on following pages	20-1
Completed Bench Sheet - A.S.U. Count	20-2
Completed Plankton Enumeration Sheet for Waterworks Operator - A.S.U. Count	20-3
Completed Plankton Analysis Card (2 sided) for MOE Lab. staff - A.S.U. Counts	20-4
Completed Bench Sheet - Volumetric Count	20-5
Completed Plankton Analysis Card Volumetric Count	20-6

MICROSCOPE CALIBRATION DATA

* 1MM = 1000 MICROMETERS

COMPOUND MICROSCOPE NO. _____

***WIDTH OF S-R CELL / WIDTH OF 1 STRIP

= .20 mm / .062 mm

	OBJECTIVE	Linear Dimensions of Whipple Square in Millimeters * WHOLE	LARGE	SMALL	Width of Entire Field	Factor for Conversion to Count/mL (1 S-R STRIP)
OCULAR	10x	.700	.070	.014	1.240	
10x	20x	.350 mm / 350 µm	.035 / 35	.007 / 7	.620 / 620	32.28 **
	40x	.176	.0175	.0035	.320	
	100x					

*** AREA OF CHAMBER / AREA OF RADIUS

= 12.5*12.5*3.14159 / 12.5 * .230 mm

INVERTED MICROSCOPE NO. _____

	OBJECTIVE	WHOLE	LARGE	SMALL		(1 RADIUS) Utermohl Chamber
OCULAR	10x	.450	.045	.009	.910	
10x	20x	.222	.022	.0045	.455	
	40x	.110 mm / 110 µm	.011 / 11	.0022 / 2.2	.230 / 230	170.65173 ***
	100x					

BENCH SHEET - PLANKTON ENUMERATION

Source (Lake): Lake Simcoe — Date Analyzed: July 30/91 — Sample No. 91-998-A

Location (Station): Brechin W.W. — Counted By: G. Hopkins — Date Sampled: June 25/91 — P 1 of 1

Depth: Raw H2O — Count Procedure: 2 strips 15-R, 1 strip* — Micr. Factor: 16.13 — Conc. (f) 1 x 2mL x 400 = .02 01625 (combined factor)

Taxonomic Name		Total 3g. microns	Total per mL A.S.U.
Sphaerocystis*	8d - III, 5d - ++++ II, 3d - ++++ ++++ IIII, 4d - IIII, 7d - +++ 629.88 x 2 =	(33) 1259.76	25.40
Dinobryon	(10x6)" (7x5)" (7x4)++++ I 7d - I (8x4)" (6x4)" (10x3)" (8x5)' (9x3)' (14x5)" 5d - I	(24) 897.11	18.08
Unid chrysophyte	3d - ++++ ++++ I 7d - III 4d - +++ 5d - III	(22) 314.95	6.35
Chlamydomonas	(7x6)" (5x4)++++ (6x5)++++ (10x8)' (7x5)' (9x7)' (12x10)' (10x9)" (4x3)'	(20) 734.00	14.80
Nephrocytium	(10x4)++++ III	(1) 320.00	6.45
Unid armoured	Dinophyte (50x35) (20x12) (23x6)	(5) 2128.00	42.90
Rhodomonas	8d - I 10d - II	(3) 207.35	4.18
Cryptomonas	(5x7)" (6x7)'	(3) 112.00	2.26
Epithemia	(3x7)++++ I (4x9)" (2x5)' (3x8)" (2x6)"	(15) 316.00	6.37
Asterionella formosa	(80x3) (70x3)	(2) 450.00	9.07
Oocystis	4d - I 3d - ++++ ++++ +++, 5d - I 3d - III 9d - I	(21) 223.08	4.50
Chroococcus	5d - I	(1) 19.63	0.40
Chromulina	8d - ++++	(5) 251.35	5.07
Anabaena	(7x6) (20x6) (30x6) (14x6) (10x6) (18x6) (7x6)	(7) 636.00	12.82
Coelosphaerium	20d	(0) 314.00	6.33
Ceratium hirundinella	[(136x8)+(90x6)+(68x9)+(68x9)+(95x33)]IIII (15-R 15988) ÷1613	(4) = 991.20	19.99
Nitzschia	(45x2)	(1) 90.00	1.81
Also present:	Navicula, Pediastrum, Fungal spores		
Protozoa	Ciliates IIII, Rotifers - Keratella', Polyarthra"		

Check 9264.43 g Microns = 186.79

Total per mL A.S.U. = 186.78

20-2

SUMMARY SHEET - PLANKTON ENUMERATION

Municipality: MARA TWP. - BRECHIN W.W Source: Lake Simcoe Sample No. 91-998-A

Sampling Point (Raw Water etc.): Raw H_2O Date Sampled June 25/91

Analyzed by: G. Hopkins Date Analyzed July 30/91

Enumeration Procedure Use: 2 strips, 1 strip*, 1 S-R Concentration Factor 2

Enumeration Factor - Algae: 16.13 (0.02) Enumeration Factor - Zooplankton 16.13

ALGAE Taxonomic Group	Total Sq. Microns	A.S.U. Per ml.	
Blue Greens			
Chroococcus	19.63	(p) 0.4	
Anabaena	636.00	13	
Coelosphaerium	314.00	6	
B.G. Total			19
Greens			
Sphaerocystis *	1259.76	25	
Nephrocytium	320.00	6	
Oocystis	223.08	5	
Pediastrum	–	p	
Green Total			36
Chlamydomonas	734	15	
Flagellates Dinobryon	897.11	18	
Unid. chrysophyte	314.95	6	
Chromulina	251.35	5	
Unid. armoured Dinophyte	2128.09	43	
Ceratium hirundinella	(1.5g) 991.2	20	
Rhodomonas	207.35	4	
Cryptomonas	113.00	2	
Flag. Total			113
Diatoms			
Epithemia	316.00	6	
Asterionella formosa	450.00	9	
Nitzschia	90.00	2	
Navicula	–	p	
Diatom Total			17
TOTAL A.S.U. PER ML.			185

ZOOPLANKTON Taxonomic Group	Total Pieces	No. per ml.
PROTOZOA		
Ciliates	4	32
Flagellates		
Sarcodina		
Total No. Per ml.		32
MICRO-INVERTEBRATES		
Rotifers		
Keratella	1	8
Polyarthra	2	16
Total No. Per ml.		24
CRUSTACEA		
Cyclops		
Daphnia		
Total No. Per ml.		
MISCELLANEOUS		
Fungal spores		p
Total No. Per ml.		
Total No. ZOOPLANKTON Per ml.		56

DIAGRAM - UNKNOWNS

WATERWORKS INFORMATION

Raw H_2O Temp. 19.5°C

Raw H_2O Turbidity

Raw H_2O Odour (if present)

Threshold Odour No.

Microstrainers: in/out of service

Weather: (if unusual and affecting intake)

Air Temp.

Filter runs: hrs.

Remarks: p = present at < 0.5 A.S.U/ml

MOE 0888 3/77

PLANKTON ANALYSIS

FILE NUMBER: 3-3-12-2

SAMPLE NUMBER: 91-998-[redacted]

Municipality: MARA TWP. - BRECHIN W.W. | Date Analysed: July 30/91 | Date Sampled: June 25/91

Source: Lake Simcoe | Enumeration Procedure: 2 strips, 1 strip*, 1 S-R

Station: Raw H2O | Depth: 4.0 m | Mic Factor: 16.13 (.02) | Concentration Factor: 1x2=2

CYANOPHYCEAE		A.S.U.
Chroococcus		p
Anabaena		13
Coelosphaerium		6
TOTAL →		19

DINOPHYCEAE		A.S.U.
Unid. armoured Dinophyte		43
Ceratium hirundinella		20
TOTAL →		63

CRYPTOPHYCEAE		A.S.U.
Rhodomonas		4
Cryptomonas		2
TOTAL →		6

EUGLENOPHYCEAE		A.S.U.
TOTAL →		Ø

CHRYSOPHYCEAE		A.S.U.
Dinobryon		18
Unid. chrysophyte		6
Chromuline		5
TOTAL →		29

CHLOROPHYCEAE		A.S.U.
Sphaerocystis	*	25
Chlamydomonas		15
Nephrocytium		6
Oocystis		5
Pediastrum		p
TOTAL →		51

BACILLARIOPHYCEAE		A.S.U.
Epithemia		6
Asterionella formosa		9
Nitzschia		2
Navicula		p
TOTAL →		17

TOTAL AREAL STANDARD UNITS PER ML → 185

OR ~~TOTAL CYSTS/ANIMALS PER ML~~

ZOOPLANKTON	No. Organisms per ML
Protozoa - ciliates	32
Rotifers - Kentella	8
- Polyarthra	16
Fungal spores	p.
TOTAL →	56

REMARKS: p = present at < 0.5 ASU/mL

Raw H2O temp. = 19.5°C

SIGNED: G. Hopkins

MOE 0899 6/82

BENCH SHEET - PLANKTON ENUMERATION

Source (Lake): Lake Simcoe | Date Analyzed: July 30/91 | Sample No. 91-998-V

Location (Station): Brechin W.W. | Counted By: G. Hopkins | Date Sampled: June 25/91 | p 1 of 1

Depth: Raw H_2O | Count Procedure: 10 radii, 5 pr* W.C. | Micr. Factor: 17.065/173 = .000853258 | Conc. (F) 10 x 2 mL x 1000 (combined factor)

Taxonomic Name		Total	microns	Total per mL / cu. mic
Sphaerocystis	* $(8^s)^{II}$ 1 $(5^s)^{IIII}$ 8 · 1 · $(3^s)^{III}$ 1 · 8 · 1 · 1 · $(4^s)^{IIII}$ 1 · 8 · $(7^s)^{III}$ 1 · * ÷5 x 10 3114.92	= (33)	622984	* 5.32
Dinobryon	$(10x6^d)^{II}$ $(7x5^d)^{II}$ $(7x4^d)^{III}$ $(7^s)^{I}$ $(8x4^d)^{III}$ $(6x4^d)^{II}$ $(10x5^d)^{II}$ $(8x5^d)^{I}$ $(9x5^d)^{I}$ $(14x5^d)^{II}$ (55)	(24)	3432.94	2.93
Unid. Chrysophyte	$(3^s)^{IIII}$ $(7^s)^{III}$ $(4^s)^{III}$ $(5^s)^{III}$	(22)	1058.21	.90
Chlamydomonas	$(7x6^d)^{II}$ $(5x4^d)^{IIII}$ $(6x5^d)^{IIII}$ $(10x8^d)$ $(7x5^d)$ $(9x7^d)$ $(12x10^d)$ $(10x9^d)^{III}$ $(4x3^d)$	(20)	5204.00	4.44
Nephrocytium	$(10x4^d)$ 8.	(1)	1040.00	.88
Unid. Armoured Dinophyte	(50x35x10) (20x12x6) (23x16x8)	(3)	21844.00	18.68
Rhodomonas	[1.05 (8x2x2)]' [1.05 (10x3x3)]"	(3)	222.60	.18
Cryptomonas	[4.2 (5x7x4)]" [4.2 (6x7x4)]'	(3)	1881.60	1.60
Epithemia	$(3x7^d)^{IIII}$ $(4x9^d)^{II}$ $(2x5^d)^{I}$ $(3x8^d)^{III}$ $(2x6^d)^{III}$	(15)	1872.00	1.60
Asterionella formosa	$(65x3^d)^{III}$ $(85x3^d)$ 8. 8. 8	(6)	15645.00	13.35
Oocystis	[4.2 (2^2x4)]' [4.2x(1.5^{2}x3)]^{IIII} [4.2 (5^2x7)]' [4.2 (1.5^{2}x3)]^{III} [4.2 (4^2x9)]'	(21)	1917.30	1.63
Chroococcus	$(5x3^d)^{I}$	(1)	35.00	.03
Chromulina	$(4x8^d)^{IIII}$	(5)	1000.00	.85
Anabaena	[6^dx7]" [6^dx20] [6^dx30] [6^dx19] [6^dx18] [6^dx10]	(7)	2968.00	2.53
Coelosphaerium	$(3x2^d)$ .34	(1)	306.00	.26
Ceratium hirundinella	W.S. = whole chamber factor = .00005 [(1.05x136x8^2)+(1.05x90x6^2)+(1.05x68x9^2)+(1.05x68x4^2)+(45x33x22)] 57,881.85 IIII	(4)	12106.87 (207,527.40)	W.C. 10.38
Nitzschia	(45x2x2)	(1)	180.00	.15
Also present:	Navicula, Pediastrum, Fungal Spores			
Protozoa	Ciliates IIII, Rotifers - Keratella', Polyarthra"			

Check 76,943 · 36 Micron = 65.65 $\mu m^3 \times 10^3$

Total per mL μm^3 - 65.71 $\times 10^3$

20-5

PLANKTON ANALYSIS

FILE NUMBER: 3-3-12-2

SAMPLE NUMBER: 91-998-

Municipality: MARA TWP. - BRECHIN W.W. Date Analysed: July 30/91 Date Sampled: June 25/91

Source: Lake Simcoe Enumeration Procedure: 10 radii, 5 r*, W.C.

Station: Raw H2O Depth: 4.0 m. Mic Factor: 17.065173 Concentration Factor: 10 x 2 = 20

CYANOPHYCEAE		$\mu^3 \times 10^{-3}$
Chroococcus	p.	
Anabaena	3	
Coelosphaerium	p.	
TOTAL →		3

DINOPHYCEAE		$\mu^3 \times 10^{-3}$
Unid. armoured Dinophyte		19
Ceratium hirundinella		10
TOTAL →		29

CRYPTOPHYCEAE		$\mu^3 \times 10^{-3}$
Rhodomonas	p	
Cryptomonas	2	
TOTAL →		2

EUGLENOPHYCEAE		$\mu^3 \times 10^{-3}$
TOTAL →		Ø

CHRYSOPHYCEAE		$\mu^3 \times 10^{-3}$
Dinobryon		3
Unid. Chrysophyte		1
Chromulina		1
TOTAL →		5

CHLOROPHYCEAE			$\mu^3 \times 10^{-3}$
Sphaerocystis	*		5
Chlamydomonas			4
Nephrocytium			1
Oocystis			2
Pediastrum		p	
TOTAL →			12

BACILLARIOPHYCEAE		$\mu^3 \times 10^{-3}$
Epithemia		2
Asterionella formosa		13
Nitzschia		p
Navicula		p
TOTAL →		15

ZOOPLANKTON	No. Organisms per ML
Protozoa - ciliates	3
Rotifers - Keratella	1
- Polyarthra	2
Fungal spores	p
TOTAL →	6

REMARKS: p = present at < 0.5 $\mu^3 \times 10^3$/mL

Raw H2O temp = 19.5°C

~~TOTAL AREAL STANDARD UNITS PER ML~~

OR TOTAL CUBIC MICRONS PER ML → 66×10^3

SIGNED: S. Hopkins

MOE 0809 6/82